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THE POTENTIATION OF NEURONAL RESPONSES TO THE MONOAMINES BY TRICYCLIC ANTIDEPRESSANT DRUGS : A MICROELECTROPHORETIC STUDY

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SUMMARY

This thesis describes some experiments performed using the technique of microelectrophoresis. The object of the experiments was to examine the suggestion that potentiation of responses to monoamines by tricyclic antidepressant drugs resulted from the blockade of monoamine uptake into presynaptic terminals.

1. The sensitivity of single neurones to microelectrophoretically applied dopamine, noradrenaline, 5-hydroxytryptamine and acetylcholine was investigated in the caudate nucleus of the rat, anaesthetised with halothane.

   a) Both excitatory and depressant responses to each of the agonists could be observed. There was a high correlation between the direction of responses to dopamine and noradrenaline, whereas there was no significant correlation, either positive or negative, between the direction of responses to dopamine and acetylcholine.

   b) The effect of desipramine was studied on both excitatory and depressant responses to dopamine, noradrenaline and 5-hydroxytryptamine, and on excitatory responses to acetylcholine. Both potentiation and antagonism of neuronal responses to monoamines and acetylcholine could be observed after a brief application of desipramine.

c) Excitatory responses to glutamate were not affected by desipramine.

d) The observation that responses to dopamine and noradrenaline can be potentiated by desipramine in the caudate nucleus cannot be explained on the basis of uptake blockade, since data in the literature show that desipramine does not block the uptake of catecholamines in the caudate nucleus.

2. The technique of microelectrophoresis was used in order to study the effects of iprindole on single neurones in the cerebral cortex and caudate nucleus of the rat.

a) Both potentiation and antagonism of neuronal responses to noradrenaline, dopamine and 5-hydroxytryptamine could be observed after a brief application of iprindole. Potentiation and antagonism often occurred after the same application of iprindole, antagonism always preceding potentiation.

b) Responses to acetylcholine were affected by iprindole similarly: both potentiation and antagonism of the responses could be observed.

c) Responses to glutamate were not affected by iprindole.

d) It is concluded that the potentiation of responses to monoamines by iprindole cannot be explained on the basis of uptake blockade, since data in the literature show that iprindole does not block the uptake of monoamines into brain tissue.

3. The technique of microelectrophoresis was used in order to study the effects of desipramine on responses of single cortical neurones to mescaline.

a) Both potentiation and antagonism of neuronal responses to mescaline could be observed after a brief application of desipramine.

b) Responses to glutamate were not affected by desipramine.

4. Biochemical techniques were used to study the uptake of radioactively-labelled noradrenaline and mescaline into synaptosomes prepared from rat cerebral cortex.

a) Mescaline is accumulated into synaptosomes by a temperature and sodium-dependent process.

b) The uptake of mescaline is not affected by desipramine.

c) The uptake of noradrenaline is inhibited by mescaline in a non-competitive manner.

d) It is concluded that the uptake of mescaline is not brought about by the noradrenaline uptake

mechanism.

e) It is concluded that the potentiation of neuronal responses to mescaline by desipramine cannot be explained by the blockade of mescaline uptake.

5. a) It is suggested that uptake blockade is not a prerequisite for the potentiation of neuronal responses to the monoamines by tricyclic antidepressant drugs.

b) It is proposed that potentiation may be due to the blockade of masked, functionally opposite, receptors on the postsynaptic cell.

c) It is suggested that the common pharmacological action of the tricyclic antidepressant drugs may be the ability to block both monoamine and acetylcholine receptors in the brain.

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CONTENTS

CHAPTER I

INTRODUCTION

1:1 OBJECT OF STUDY

Tricyclic antidepressant drugs have been shown to potentiate responses to exogenously applied monoamines in peripheral and central pharmacological test systems (Gyermek & Possemato, 1960; Bradshaw, Roberts & Szabadi, 1974). Furthermore, it has been demonstrated that the tricyclic antidepressant drugs block the uptake of monoamines into presynaptic terminals (Iversen, 1965; Ross & Renyi, 1969). A causal relationship between these two effects is assumed by the uptake blockade hypothesis of potentiation (Iversen, 1974). According to this hypothesis, the blockade of monoamine uptake results in an increased monoamine concentration at receptors which, in turn, is reflected in an enhanced response to the monoamine.

The experiments described in this thesis were conducted in an attempt to test the uptake blockade hypothesis of potentiation. The microelectrophoretic technique was used to determine the effect of tricyclic antidepressant drugs on responses of single neurones to monoamines in situations where uptake blockade was unlikely to contribute to the effects observed.

Thus, the effect of desipramine on responses of single caudate neurones to noradrenaline (NA), 5-hydroxytryptamine (5HT) and dopamine (DA) was examined because desipramine has been shown not to block the uptake of these amines into nerve terminals in the caudate nucleus (Ross & Renyi, 1967, 1969). Similarly, iprindole has been shown to have no effect on the uptake of monoamines into nerve terminals in

both the cerebral cortex and the caudate nucleus (Ross, Renyi & Ogren, 1971). Therefore, the effect of iprindole on responses of single neurones in the cerebral cortex and caudate nucleus to NA, 5HT and DA was examined.

Finally, it has been reported that mescaline can mimic the actions of other monoamines when applied by microelectrophoresis to single cortical neurones (Bevan, Bradshaw, Roberts & Szabadi, 1974a), and it has been reported that mescaline has a very low affinity for NA-uptake mechanisms in peripheral adrenergic nerves (Iversen, 1967). Thus the effect of desipramine on responses of single cortical neurones to mescaline was examined. As there have been no data, however, concerning the uptake of mescaline into brain tissue, the uptake of mescaline into synaptosomes prepared from rat brain cerebral cortex was also examined.

1:2 MONOAMINE THEORY OF AFFECTIVE DISORDERS

Contemporary research interest in the monoamines and the affective disorders was stimulated by the discovery that iproniazid, a monoamine oxidase inhibitor, was also a therapeutically effective antidepressant agent (Crane, 1957; Loomer, Saunders & Kline, 1957). Reserpine was used in small doses to treat hypertension (Kline, 1959), but often produced severe depressive reactions (Muller, Pryor, Gibbons & Orgain, 1955; Harris, 1957).

The common denominator in the action of these drugs appeared to be an interaction with the metabolism of the monoamines noradrenaline (NA), 5-hydroxytryptamine (5HT) and dopamine (DA). Reserpine depleted the brain of these monoamines (Shore, 1962) whereas iproniazid tended to elevate monoamine levels (Schildkraut, 1965). The fact that these drugs could either produce or relieve depression whilst altering monoamine levels in opposite ways suggested that perhaps both mania and depression may be caused by alterations in monoamine metabolism (Jacobsen, 1964).

The monoamine theory of affective disorders proposes that impaired function of monoamine containing neuronal systems forms the basis of the affective disorders: decreased monoamine transmission from monoamine neurones leads to symptoms of depression whereas increased transmission from monoamine neurones leads to symptoms of mania (Schildkraut, 1965; Davis, 1970). This theory is, however, derived from the rather circumstantial evidence that drugs which influence mood, drive and motivation

change presynaptic biochemical events in a manner which can lead to predictions about changes in synaptic transmission (Davis, 1970). More recently, changes in the sensitivity of the postsynatpic membrane to the neurotransmitter have been implicated in the aetiology of affective disorders (MRC, 1972).

It has been proposed that the blockade of monoamine uptake into nerve terminals by tricyclic antidepressant drugs results in an increase in the monoamine concentration at the postsynaptic receptor which, in turn, is reflected in an enhanced effect of the monoamine (Iversen, 1965). It is assumed that this potentiation of monoamine effects is responsible for the clinical effectiveness of imipramine and desipramine in the treatment of depressive episodes (Schildkraut, 1965). It has been suggested that the efficacy of tricyclic antidepressant drugs in alleviating depression reflects the ability of these drugs to inhibit the accumulation of monoamines into nerve terminals (Iversen, 1974).

The pharmacology of the tricyclic antidepressant drugs imipramine, desipramine and iprindole will therefore be described with respect to their effects on monoamine responses and their effects on the uptake of monoamines into nerve terminals.

1:3 THE PHARMACOLOGY OF TRICYCLIC ANTIDEPRESSANT DRUGS

According to Haflinger (1959), iminodibenzyl, the parent compound of imipramine-like drugs, was synthesised in 1899 by Thiel and Holzinger. Little attention was paid to its pharmacology until 1948 when a number of derivatives were synthesised as potential antihistaminic agents (Schindler & Haflinger, 1954). However, because of the structural similarity to the phenothiazines, one derivative, imipramine, was tested on schizophrenic patients. There was no evidence of antipsychotic action, but Kahn (1957; 1958) noted an elevation in the mood of patients.

Early studies on the metabolism of imipramine resulted in the isolation of an N-demethylated derivative desmethylimipramine (desipramine) by Herman and Pulver (1960). It was subsequently believed for some time that desipramine was responsible for the clinical effectiveness of imipramine (Brodie, Bickel & Sulser, 1961; Gillette, Dingell, Sulser, Kuntzman & Brodie, 1961) but it was later shown that imipramine did have a clinical antidepressant action of its own (Hollister, Overall, Johnston, Katz, Kimbel & Honigfeld, 1963; diMascio, Heninger & Klerman, 1964).

Iprindole, a clinically effective antidepressant drug (Hicks, 1965; Johnson & Maden, 1967), differs from the imipramine-type antidepressants in being an indole with an eight-membered saturated third ring. This structural change introduces pharmacological changes of particular interest (see below).

1:3,1 THE PERIPHERAL PHARMACOLOGY OF IMIPRAMINE AND DESIPRAMINE

1:3.1.1. Interaction with monoamines and acetylcholine in smooth muscle test systems

Both imipramine and desipramine potentiated the response to exogenously applied NA of the cat nictitating membrane (Sigg, 1959; Soffer & Gyermek, 1961; Sturman, 1971). In larger doses, however, potentiation was not seen; instead the responses to NA were antagonised by imipramine and desipramine (Sigg, 1959; Schaeppi, 1960). The potentiation of the response to NA was shown not to be due to circulating catecholamines being released from the adrenal medulla (Domenjoz & Theobald, 1959; Gyermek & Soffer, 1961; Soffer & Gyermek, 1961).

Although imipramine itself has almost no direct vasopressor action (Ryall, 1961; Cairncross, Gerschon & Gus, 1963), the vasopressor action of NA could be potentiated by imipramine in the carotid and femoral arteries of the cat and of the dog (Osborne & Sigg, 1960; Osborne, 1962). In the carotid artery of the cat, for example, low doses (75 µg/kg) of imipramine potentiated the vasopressor responses to NA in vivo while high doses (15 mg/kg) markedly diminished the vasopressor responses (Osborne & Sigg, 1960).

Turker and Khairallah (1967) showed that desipramine could potentiate the contractile response of aortic strips to NA whereas increasing the concentration of desipramine abolished the response to NA. Toda (1971) noted that the NA-induced contraction of pulmonary artery,

as well as of aortic, strips was potentiated by 2.10^{-7}M desipramine, but antagonised by 10^{-6}M desipramine. Hedina & Ling (1970) also reported a noradrenolytic effect in their investigation of the interaction between desipramine and the NA-induced contraction of the isolated perfused renal artery. Desipramine decreased both the maximum and the slope of the log-dose-response curve to NA.

In the rabbit ear artery preparation, a low dose of desipramine (10^{-8}M) potentiated the response to NA whereas a higher dose of desipramine (10^{-6}M) abolished the responses (Bassett, Cairncross, Hacket & Story, 1969; McCulloch & Story, 1972). Similar results have been reported from studies in the vas deferens of the rat. Desipramine potentiated the response of the vas deferens to NA whereas at higher concentrations (10^{-5}M), desipramine antagonised the response (Westfall, 1973).

The effect of tricyclic antidepressants has also been studied on the responses of smooth muscle preparations to electrical stimulation of the sympathetic innervation.

Imipramine, in doses of 150-600 mg/kg, enhanced the nictitating membrane contractions in response to stimulation of the preganglionic cervical sympathetic nerve (Sigg, Soffer & Gyermek, 1963). However, Osborne and Sigg (1960), using a dose range of 50-1000 mg/kg imipramine, could observe only a diminished electrically evoked contraction of the nictitating membrane. Similarly, Costa, Garattini and Valzelli (1960) and Schaeppi (1960) could not observe potentiation of the nictitating membrane response to electrical stimulation following imipramine.

Later, however, Haefely, Hurlimann and Thoenen (1964) and Sturman (1971) confirmed the observation of Sigg et al, (1963) and reported that both imipramine and desipramine were capable of potentiating the effects of electrical stimulation of the sympathetic innervation of the nictitating membrane. Similarly, in rabbit aortic strips, transmural stimulation elicited a response which was potentiated by low doses of desipramine (2.10^{-7} M) but antagonised by higher doses (10^{-6} M) of desipramine (Toda, 1971).

Imipramine and desipramine also modify responses of smooth muscle preparations to 5HT and DA. Gyermek and Possemato (1960) observed that responses of the cat nictitating membrane to 5HT could be potentiated by imipramine. Similarly, Turker and Khairallah (1967) showed that desipramine could potentiate the responses of aortic strips to 5HT. Furthermore, increasing the concentration of desipramine abolished the responses to 5HT (Turker & Khairallah, 1967). It has been reported that both the pressor and depressor responses to DA in vivo are potentiated by desipramine in the carotid arteries of the cat (Basset et al, 1969; Kadziewala & Popielarski, 1971), guinea pig, rabbit (Kadziewala & Popielarski, 1971) and dog (Eble, 1964).

In an attempt to separate presynaptic and postsynaptic components of the interaction between tricyclic antidepressant drugs and monoamine responses, many authors have used smooth muscle preparations which have been sympathetically denervated.

Gyermek and Possemato (1960) were the first to observe that, in a sympathetically denervated preparation of

the cat nictitating membrane, imipramine failed to potentiate the response to NA. Similarly, Sturman, (1971) also reported that, in the denervated cat nictitating membrane, both imipramine and desipramine failed to potentiate the effects of NA. However, the contractile response of the nictitating membrane to NA could still be antagonised by imipramine and desipramine following denervation (Sturman, 1971). In the rabbit ear artery preparation, imipramine (10^{-9}M) potentiated the contractile response to NA. However, in the denervated preparation the same concentration of imipramine antagonised the response to NA instead (Callingham, 1967). In agreement with Callingham (1967), McCulloch and Story (1972) reported that, following denervation, only antagonism of the response to NA could be seen; potentiation was never observed. Identical results have been reported in the postganglionically denervated rat vas deferens (Westfall, 1973).

It appears that denervation does not influence the interaction between tricyclic antidepressant drugs and responses to 5HT. Gyermek and Possemato (1960) have shown, in a sympathetically denervated preparation of the cat nictitating membrane, that, whereas the potentiation by imipramine of the response to NA disappeared, the response to 5HT could still be potentiated by imipramine.

It is well documented, then, that imipramine and desipramine have a dual effect on responses to monoamines of smooth muscle preparations; both potentiation and antagonism can be observed. Moreover, the dual effect of tricyclic antidepressant drugs on monoamine responses is dose-

dependent: lower doses potentiate and higher doses antagonise the response.

Turker and Khairallah (1967) concluded that the inhibition of NA and 5HT responses could be explained on the basis of a competitive α-adrenergic blocking action of desipramine. Scriabine (1969), however, proposed that desipramine could compete with NA for both α- and β-adrenergic receptors. In contrast, Hrdina and Ling (1970) concluded that the antagonism of NA responses by desipramine was not competitive in nature. They suggested that the action of desipramine could be due to an interference with the action of calcium in initiating and maintaining the contraction of smooth muscle (Hrdina & Ling, 1970).

It is more difficult, however, to interpret the potentiation of monoamine responses. In the denervated smooth muscle preparation, potentiation of responses to NA by imipramine (Gyermek & Possemato, 1960) or desipramine (Callingham, 1967) was never observed: antagonism only was seen. It is likely, therefore, that an interaction between the tricyclic antidepressant drugs and a presynaptic process mediates the potentiating effect. The inhibition of monoamine uptake into nerve terminals by tricyclic antidepressant drugs (see below) may therefore be responsible for the potentiation of monoamine responses. However, even in the denervated smooth muscle preparation, responses to 5HT can be potentiated by imipramine (Gyermek & Possemato, 1960). It appears then that inhibition of monoamine uptake into nerve terminals is not a complete explanation for potentiation.

There is also evidence that tricyclic antidepressant drugs interact, not only with monoamine responses but with cholinergic responses as well (Domenjoz & Theobald, 1959). A small dose of imipramine (1-3 mg/day) in cats diminished the bradycardia which followed the stimulation of the peripheral cut vagus nerve (Sigg, 1959) and reduced the pilocarpine induced salivation (Sigg, 1959; Sulser, Watts & Brodie, 1961). Furthermore, in the isolated intestine, imipramine blocked the effect of acetylcholine (ACh) (Sigg, 1959). Osborne and Sigg (1960) reported that the hypotensive action of ACh, and of peripheral stimulation of the cut vagus nerve, on the blood pressure of cats was potentiated by a low dose of imipramine (320 µg/kg). However, higher doses decreased and finally blocked the response to vagal stimulation, although the response to ACh was unaffected. Similarly, imipramine and desipramine (0.033-0.16mM) when applied to the rat phrenic nerve-diaphragm preparation, first enhanced, then depressed and finally abolished the contractile response to stimulation (Change & Chaung, 1972). The anticholinergic activity has been assessed quantitatively on the guinea pig ileum (Theobald, Buch, Kunz, Morpurgo, Stenger & Wilhelmi, 1964) and rat stomach fundal strip (Atkinson & Ladinsky, 1972) preparations.

There is evidence, then, to suggest that imipramine and desipramine may have a dual effect on responses to ACh of peripheral test systems: both potentiation and antagonism can be observed. Moreover, it is possible that the dual effect of tricyclic antidepressant drugs on responses

to ACh is dose -dependent: lower doses potentiate whereas higher doses antagonise the response.

The antagonism of responses to ACh by imipramine and desipramine has been interpreted as an antimuscarinic action of these drugs (Domenjoz & Theobald, 1959; Atkinson & Ladinsky, 1972). The potentiation of responses to ACh is more difficult to explain. Osborne and Sigg (1960) concluded that the potentiating effects of imipramine on responses to ACh was due to the blockade of cholinesterase, since they could demonstrate an inhibitory effect of imipramine on serum cholinesterase.

1:3.1.2. Inhibition of monoamine uptake

The effect of tricyclic antidepressant drugs on catecholamine uptake into nerve terminals has been studied mainly in the perfused heart preparation whereas the interaction with indolamine uptake has been studied using blood platelets.

Following an intraperitoneal injection, Herrting, Axelrod and Whitby (1961) noticed that imipramine markedly affected the amount of ^{3}H-NA which accumulated in the heart, as well as in other tissues. Slices of heart were shown to accumulate ^{3}H-NA by a process which obeyed Michaelis-Menten kinetics (Dengler, Spiegel & Titus, 1961; Dengler, Michaelson, Spiegel & Titus, 1962). The uptake of ^{3}H-NA into such slices was inhibited by imipramine and desipramine (Titus & Spiegel, 1962). Similarly, the isolated perfused rat heart accumulates NA by an active process. The NA is accumulated into nerve terminals; this uptake process is competitively inhibited by desipramine and

imipramine (Bergen & Iversen, 1965; Iversen, 1965). A desipramine concentration of $1.3.10^{-8}$M is sufficient to produce a 50% reduction in the accumulation of NA (Iversen, 1965). The accumulation of ^{3}H-NA into isolated rat atria and into the perfused guinea pig heart is also inhibited by desipramine (Leitz & Stefano, 1970). It has been demonstrated recently that the inhibition by desipramine of ^{3}H-NA accumulation into adrenergic nerves of the rabbit aorta is competitive in nature (Maxwell, Ferris, Burcsu, Woodward, Tang & Williard, 1974). Imipramine and desipramine also competitively inhibit the uptake of ^{3}H-NA into cat spleen (Herrting et al, 1961; Dengler et al, 1961) and rat vas deferens (Haggendal & Hamberger, 1967). Tuck, Hamberger and Sjoqvist (1972), incubated rat irises in the plasma from patients receiving imipramine therapy and found a reduction in the accumulation of ^{3}H-NA with respect to controls.

The uptake of 5HT into blood platelets is a high affinity sodium dependent process (Born & Gillson, 1959; Stacy, 1961; Sneddon, 1969). Furthermore, the uptake of 5HT into blood platelets is followed by an intracellular storage of the amine in reserpine-sensitive storage vesicles (Pletscher, 1968).

The uptake of 5HT into rabbit blood platelets is inhibited by imipramine and desipramine (Siva-Sankar, Borewka & Polinsky, 1964). There is some inconsistency in the literature, however, concerning the potency of tricyclic antidepressants in inhibiting the uptake of 5HT into platelets. For example, Buczko, de Gaetano and Garattini

(1974) reported that a desipramine concentration in the order of 10^{-5}M was required to reduce the uptake of ^{3}H-5HT by half, whereas Stacy (1961) reports a figure of 5.10^{-7}M. Lingjaerde (1970) reported a 91% inhibition of 5HT uptake by an imipramine concentration of 10^{-6}M. Tuomisto (1974) credits these differing values of the antidepressant concentration to poor methodology of earlier studies and reported that a 50% inhibition of 5HT uptake was obtained with concentrations of 10^{-7}M imipramine and 10^{-6}M desipramine. The inhibition was competitive in nature (Tuomisto, 1974).

The uptake of 5HT into human blood platelets is also inhibited by imipramine (Marshall, Stirling, Tait & Todrick, 1960; Yates, Todrick & Tait, 1964; Todrick & Tait, 1969; Ahtee & Saarnvaara, 1971) and desipramine (Yates et al, 1964; Todrick & Tait, 1969; Ahtee & Saarnvaara, 1971).

It has also been demonstrated that imipramine effectively blocks 5HT uptake in the rat heart (Iversen, 1965) and aortic strips (Maxwell, Keenan, Chaplin, Roth & Eckhardt, 1969).

There is evidence, then, that monoamines are accumulated into sympathetically innervated tissues and blood platelets by an active process. This active process is competitively inhibited by desipramine and imipramine.

1:3.2 THE CENTRAL PHARMACOLOGY OF IMIPRAMINE AND DESIPRAMINE

1:3.2.1. Interaction with reserpine and amphetamine

The effects of tricyclic antidepressant drugs on the central nervous system have, largely, been obtained by studying the interaction of the tricyclic antidepressant

drugs with reserpine and amphetamine effects.

As reserpine can produce severe depressive reactions in man (Miller, 1955; Harris, 1957), reserpine, and reserpine-like drugs were used extensively in animals to produce a model condition of depression which is amenable to the testing of potential antidepressant drugs.

In animals, reserpine treatment results in ptosis or blepharospasm and this can be transformed into a quantitative bioassay (Rubin, Malone, Waugh & Burke, 1957). Reserpine treatment also results in sedation; there is a decrease in locomotor activity and catatonia results (Garattini & Jori, 1967). Imipramine prevents the ptosis induced by reserpine in cats (Garattini, Giachetti, Pieri & Re, 1960). Furthermore, the sedative effect of reserpine in rats was also prevented by imipramine and desipramine (Domenjoz & Theobald, 1959; Sulser, Watts & Brodie, 1960; Cheu & Bohner, 1961). The antagonism of the sedative action of reserpine by imipramine and desipramine has also been demonstrated in mice (Askew, 1963; Aceto & Harris, 1965) and rabbits (Maxwell & Palmer, 1961).

The interaction between tricyclic antidepressant drugs and reserpine has also been determined by measuring the temperature changes induced by reserpine in rats. When reserpine is given intravenously to rats it produces changes of body temperature in two phases (Garattini & Jori, 1967). An initial phase of hyperthermia lasting for about two hours, is followed by a marked hypothermia. Imipramine and desipramine enhance the hyperthermia induced by giving reserpine intravenously to rats (Jori,

Paglialunga & Garattini, 1966). However, if given prior to reserpine, imipramine and desipramine prevent the hypothermia induced by reserpine (Garattini, Giachetti, Jori, Pieri & Valzelli, 1962). In addition, a marked increase in temperature was noted when imipramine was given to reserpinised rats in the hypothermic phase (Garattini & Jori, 1967).

In contrast to reserpine, amphetamine stimulates locomotor activity and produces hyperthermia. Imipramine and desipramine potentiate the effect of amphetamine on motor activity in rats (Furginele, Amiente & Horovitz, 1964). In addition, the amphetamine-induced hyperthermia in rats and rabbits is potentiated by imipramine and desipramine (Morpurgo & Theobald, 1965).

In addition to its effects on motor activity, amphetamine also produces behavioural arousal (Stein & Sifter, 1961). In an operant conditioning regime, rats were rewarded with hypothalamic stimulation for pressing a lever. Amphetamine enhanced this behaviour (Stein & Sifter, 1961). These authors found that imipramine prolongs and augments the rewarding effects of amphetamine with hypothalamic stimulation.

1:3.2.2. Interaction with monoamine precursors

Most studies on the interaction between tricyclic antidepressant drugs and monoamines were performed on peripheral tissues. Data regarding the interaction between tricyclic antidepressant drugs and monoamines in the central nervous system have been lacking until recently. Monoamines do not pass the blood-brain barrier and thus do

not reach central receptor sites.

The blood-brain barrier has been circumvented in studies using the monoamine precursors L-DOPA (3,4-dihydroxy-L-phenylalanine) and 5HTP (5-hydroxytryptophan). Thus it has been reported that the anti-reserpine effect of L-DOPA is potentiated by imipramine (McGrath & Ketteler, 1963).

Imipramine also potentiates the 5HTP induced fever in rabbits (Leow & Taeschler, 1965), which was interpreted as a centrally mediated action.

This interaction between tricyclic antidepressant drugs and 5HTP has also been investigated at the level of the spinal cord where Anden, Jukes and Lundberg (1964) showed that an increase in 5HT receptor activity results in the potentiation of the hindlimb-extensor reflex of the cat. Imipramine potentiated the effects of 5HTP as well as the effect of tryptophan on the extensor-hindlimb reflex (Meek, Fuxe & Anden, 1970). In a similar study, Sinclair and Sastry (1974) noted that imipramine and desipramine blocked the assumed serotinergic bulbospinal inhibition of the monosynaptic reflex in the spinal cord. Furthermore, following an infusion of either antidepressant drug, the bulbospinal inhibition was blocked, but later facilitation of the monosynaptic reflex appeared (Sinclair & Sastry, 1974).

1:3.2.3. <u>Effect on responses of single neurones to monoamines and acetylcholine</u>

The blood-brain barrier has also been by-passed by studying the interaction of tricyclic antidepressant drugs with monoamines at the level of the single neurone, using

the technique of microelectrophoresis.

In the cerebral cortex of the cat, both excitatory and depressant responses to NA were affected by both imipramine and desipramine (Bradshaw, Roberts & Szabadi, 1971a; 1974). Both potentiation and antagonism of the responses could be observed following a brief application of the antidepressant. Low doses resulted in potentiation of the responses whereas higher doses resulted in antagonism of the responses to NA (Bradshaw et al, 1974).

Avanzino, Ermirio and Zummo (1971) studied the interaction between imipramine and NA in the brain stem of the guinea pig. When imipramine and NA were applied simultaneously the size of a response to NA was larger than when NA alone was applied. Although imipramine did have a direct agonistic action on some neurones, the enhancement was interpreted as a potentiation of the NA response by imipramine. When imipramine was applied for some time before NA, no enhancement, but often a reduction, in the size of the response to NA could be observed. Desipramine has been shown to potentiate responses to NA of Purkinje cells (Hoffer, Siggins & Bloom, 1971). Similarly to NA both excitatory and depressant responses to 5HT could be potentiated and antagonised by the tricyclic antidepressant drugs (Bradshaw et al, 1971a; 1974).

The interaction between tricyclic antidepressant drugs and ACh in the central nervous system has only recently received attention. Using the microelectrophoretic technique, it was demonstrated that excitatory responses of single cortical neurones to ACh could be modified by imip-

ramine and desipramine (Bradshaw et al, 1971a; Bevan, Bradshaw, Roberts & Szabadi, 1973c; Bevan, Bradshaw & Szabadi, 1975a). As with monoamines (Bradshaw et al, 1974) both antagonism and potentiation of the responses to ACh could be observed. Antagonism of the response occurred at higher doses than potentiation (Bevan et al, 1973c; 1975a).

Thus it has been demonstrated in the central nervous system that, as in the peripheral nervous system, imipramine and desipramine have a dual effect on responses to monoamines and ACh: both potentiation and antagonism can be observed. Moreover, the dual effect of tricyclic antidepressant drugs on responses to monoamines and ACh is dose-dependent: lower doses potentiate whereas higher doses antagonise the response.

1:3.2.4. Inhibition of monoamine uptake

The interaction between tricyclic antidepressant drugs and monoamine uptake in the brain has been studied using biochemical and histochemical techniques to follow the accumulation of radiolabelled monoamines into whole brain.

Carlsson, Fuxe, Hamberger and Lindqvist (1966) determined the effect of desipramine on the accumulation of NA and DA in the brain following the administration of L-DOPA. Rats were pretreated with reserpine, to deplete monoamine stores, and with nialamide, to prevent peripheral metabolism of L-DOPA. Desipramine (10 mg/kg i.p) prevented the accumulation of NA into the brain following the administration of L-DOPA. The uptake of DA was apparently not affected. In another study, NA, DA or 5HT was injected

into the lateral ventricles of reserpine pretreated rats (Fuxe & Ungerstedt, 1968). Desipramine inhibited the accumulation of NA into nerve terminals adjacent to the ventricles. In contrast to the observations in the report of Carlsson et al, (1966), DA accumulation was also reduced whereas 5HT was not affected.

The uptake of catecholamines has been studied using both slices and synaptosomes prepared from various regions of the brain. There is a highly active catecholamine transport system into both dopaminergic neurones and into noradrenergic neurones (Snyder & Coyle, 1969). Therefore the striatum has often been used as a dopaminergic uptake system whilst the cerebral cortex or hypothalamus has been used as a noradrenergic uptake system (Ross & Renyi, 1967; 1969; Ross et al, 1971; Horn, Coyle & Snyder, 1971).

The uptake of ^{3}H-NA into slices of cerebral cortex of the mouse (Ross & Renyi, 1967) and rat (Haggendal & Hamberger, 1967; Saloma, Insalaco & Maxwell, 1971) and into slices of the midbrain of the mouse and rat (Ross et al, 1971) was inhibited by imipramine and desipramine. The inhibition was competitive in nature (Saloma et al, 1971) and was completely inhibited by an imipramine concentration of 10^{-5}M (Haggendal & Hamberger, 1967; Ross et al, 1971). Similar results have been obtained using synaptosome preparations. In the cerebral cortex, Maxwell et al (1974) have shown that desipramine competitively inhibits the uptake of ^{3}H-NA. Furthermore, desipramine and imipramine competitively inhibit the accumulation of ^{3}H-NA into hypothalamic synaptosomes.

Similar to NA, the uptake of ^{3}H-DA into hypothalamic synaptosomes was competitively inhibited by both imipramine and desipramine (Horn et al, 1971).

In contrast to their effects on catecholamine uptake in the cerebral cortex and hypothalamus, imipramine and desipramine hardly affected the accumulation of ^{3}H-NA or ^{3}H-DA into striatal synaptosomes. Imipramine was 100 times less potent, and desipramine 1000 times less potent in inhibiting the accumulation of NA and DA into striatal synaptosomes, as compared with hypothalamic synaptosomes (Horn et al, 1971). Similarly, Ross et al, (1971) and Haggendal and Hamberger (1967) reported that the uptake of ^{3}H-DA into slices of striatum was not affected by desipramine.

The uptake of ^{3}H-5HT (Ross & Renyi, 1969) and ^{14}C-5HT (Ross et al, 1971) into slices prepared from the mouse cerebral cortex was competitively inhibited by both imipramine and desipramine. Furthermore, the administration of imipramine or desipramine in vivo decreased the capacity of the tissue to accumulate ^{3}H-5HT when subsequently examined in vitro (Ross & Renyi, 1969; Carlsson, 1970). The accumulation of ^{3}H-5HT into brain slices was also inhibited when the incubations were performed using plasma from patients receiving imipramine treatment (Hamberger & Tuck, 1973). The uptake of ^{3}H-5HT into rat forebrain synaptosomes is also inhibited by imipramine (Kuhar, Roth & Aghajanian, 1972).

There is evidence then, that monoamines are actively accumulated into nerve terminals in the brain. In the

cerebral cortex, the accumulation of NA, 5HT and DA is competitively inhibited by imipramine and desipramine. Similarly, the uptake of NA and DA into nerve terminals in the hypothalamus can be inhibited by both imipramine and desipramine. In contrast, neither imipramine nor desipramine inhibited the uptake of NA and DA into nerve terminals in the caudate nucleus.

1:3.3 THE PHARMACOLOGY OF IPRINDOLE

The striking difference between the pharmacology of iprindole and other tricyclic antidepressant drugs is that iprindole is almost completely without any monoamine uptake blocking activity. For example, iprindole has little effect on the uptake of ^{3}H-NA into the heart or into brain tissue in vivo. Whereas 5 mg/kg imipramine inhibited the uptake of NA by 50% in both tissues, 50 mg/kg iprindole achieved only a 12% inhibition of NA uptake (Gluckman & Baum, 1969). Iprindole (15 mg/kg) did not alter the ^{3}H-NA accumulation into rat heart (Lemberger, Serratinger & Kuntzman, 1970); indeed, no effect on catecholamine uptake into rat heart could be detected with iprindole concentrations as high as 200 mg/kg (Lahti & Maickel, 1971). Rosloff and Davis (1974) injected ^{3}H-NA intracisternally and could not find any effect of iprindole on the accumulation of radioactivity into the rat brain. They also examined the effect of iprindole on the uptake of DA into striatal synaptosomes and the uptake of NA and 5HT into synaptosomes from the rest of the brain (whole brain minus the striatum). No effect of iprindole on the uptake of

the monoamines could be detected (Rosloff & Davis, 1974).

Like imipramine, iprindole antagonises the reserpine-induced ptosis in mice, and also reverses the reserpine-induced hypothermia (Gluckman & Baum, 1969). Iprindole also reverses the loss of appetite induced in rats by amphetamine (Gluckman & Baum, 1969) and enhances and prolongs the psychomotor stimulation produced in rats by amphetamine (Miller, Freeman, Dingell & Sulser, 1970). The pressor response of the dog femoral artery to NA is potentiated by iprindole (Gluckman & Baum, 1969). Similarly, the NA-induced increase in the blood pressure of rabbits is potentiated by iprindole, although only antagonism of the NA-induced contraction of rabbit aortic strips could be observed (Saarnvaara & Mattila, 1974). The interaction between iprindole and NA pressor effects has also been studied in humans (Fann, Davis, Janowsky, Kaufmann, Cavanaugh & Oats, 1974). In five patients diagnosed as depressed, NA-induced pressor-response curves were constructed. Iprindole, in doses of 60 mg and 120 mg, did not change the NA-induced blood pressure response (Fann et al, 1974).

The effect of iprindole on responses to ACh has received little attention. Gluckman and Baum (1969) reported that the response of the guinea pig ileum to ACh was inhibited by iprindole.

Iprindole shares with imipramine and desipramine the ability to antagonise the effects of reserpine and enhance the effects of amphetamine. Furthermore, iprindole potentiates the responses to NA of sympathetically innervated tissues. These observations are particularly interesting

as it has been shown that iprindole does not share with other tricyclic antidepressant drugs the ability to inhibit the accumulation of monoamines into nervous tissue.

1:4 MICROELECTROPHORESIS

Monoamine transmission forms the basis of many current theories of the mode of action of psychoactive drugs, including the tricyclic antidepressants (Davis, 1970), the neuroleptics (Stein, 1971) and the hallucinogens (Brawley & Duffield, 1972). Many of the interactions between psychoactive drugs and monoamine transmitters can be studied at the level of the single brain cell using the technique of microelectrophoresis.

The microelectrophoretic technique consists of the ejection into the extraneuronal space of pharmacologically active drugs as ions from glass micropipettes, and of the simultaneous recording of extracellular action potentials. The site of drug action is thus restricted to the immediate vicinity of the neurone under observation. The blood-brain barrier is, then, circumvented allowing the interaction between psychoactive drugs and putative neorotransmitters to be tested directly on single neurones in the brain.

The experiments reported in this thesis used this technique to examine the effect of tricyclic antidepressant drugs on responses of single caudate and cortical neurones to monoamines and ACh. Previous reports on the effects of microelectrophoretically applied monoamines and ACh, on single cortical and caudate neurones will be discussed below.

1:4.1 CEREBRAL CORTEX

1:4.1.1. Noradrenaline

Krnjevic and Phillis (1963) investigated the responses of cortical neurones to NA in the barbiturate-anaesthetised cat. On these cells, which were activated by L-glutamate or DL-homoscysteate, NA had a predominantly depressant action, although excitatory responses were observed when higher currents were used. Johnson, Roberts & Straughan (1969b) confirmed that, when barbiturate anaesthesia was used, the predominant effect of NA was depression, but pointed out that in encephale isole preparations cortical neurones responded to NA more frequently with excitation.

Johnson, Roberts, Sobieszek & Straughan (1969a) were careful to control for the possible effects of hydrogen ions released from the micropipette during the application of NA. They reported that NA released from solutions at pH 3 had identical effects to NA released from solutions at pH 5. They concluded that hydrogen ions were unlikely to contribute to the observed responses to NA. However, these findings, confirmed later by Stone (1972), were disputed by Fredericksen, Jordan & Phillis (1971). They argued that NA was exclusively depressant when released from solutions whose pH was greater than 4 whereas excitatory responses were encountered when solutions of pH 3 or less were used. Subsequently it was reported that hydrogen ions, released from solutions of HCl could also excite cortical neurones (Jordan, Lake & Phillis, 1972a). These authors thus argued that the true action of NA on cortical neurones is inhibitory.

The effect of NA on cortical neurones was subsequently

re-examined (Bevan, Bradshaw, Roberts & Szabadi, 1973a). The responses of cortical neurones to NA released from solutions at pH 3.1 and pH 5.0, and the effect of hydrogen ions released from HCl were compared. Each cell excited by NA (pH 3.1) was also excited by NA (pH 5.0); similarly, each cell depressed by NA (pH 5.0) was also depressed by NA (pH 3.1). One cell responded to hydrogen ion application, and that response was a depression. When released from a solution at pH 3.1, NA usually evoked a larger response than when released from solutions at pH 5.0 (Bevan et al, 1973a). This was attributed to a reduction in the transport number of NA as the pH of the solution is raised by the addition of NaOH. This was subsequently confirmed by in vitro studies (Bevan, Bradshaw, Roberts & Szabadi, 1973b).

Recent reports have also demonstrated both excitatory and depressant responses to NA in the cerebral cortex of the cat (Frederickson, Jordan & Phillis, 1972; Bevan et al, 1974a), the rat (Stone, 1973a) and the rabbit (Giardina, Pedemente & Sabelli, 1972).

Excitatory responses to NA could be antagonised by the α-adrenoceptor antagonists phentolamine, dibenamine and thymoxamine, and by the β-adrenoceptor antagonists propranolol, sotalol and isopropylaminonitrophenylethanol (INPEA) (Johnson et al, 1969a), whilst depressant responses were rarely affected. The antagonisms were specific in that excitatory responses to ACh were not affected (Johnson et al, 1969a). Frederickson et al (1972) demonstrated that depressant responses to NA could be antagonised by the adrenergic blockers dibenamine, phentolamine, phenoxybenzamine and

INPEA. Excitatory responses to NA, which these authors attributed to the depression of a nearby inhibitory neurone, were also antagonised by dibenamine, phenoxybenzamine and phentolamine. Depressant responses to NA could also be antagonised by bulbocapnine (Anderson & Stone, 1974) and also by strychnine (Yarbrough, Lake & Phillis, 1974). In an earlier report (Stone, 1973a) however, no effect of strychnine on NA depression could be found.

Recently it has been reported that responses to NA can be potentiated by sotalol (Bevan, Bradshaw & Szabadi, 1974b). Excitatory responses to NA were potentiated when the sotalol was ejected with a low current, but potentiation was superseded by antagonism when the intensity of the ejecting current was increased. Excitatory responses to NA were affected in a similar manner by methysergide: both potentiation and antagonism could be observed (Bevan et al, 1974b). Responses to ACh were not affected by sotalol or methysergide.

Cyclic AMP, and dibutyryl cyclic AMP, had either no effect or only weak depressant action on cortical neurones which were depressed by NA (Jordan, Lake & Phillis, 1972b; Lake, Jordan & Phillis, 1973). Phosphodiesterase inhibiting drugs, such as aminophylline and papaverine, enhanced the depression produced by NA, but were reported to have depressant actions of their own (Lake et al, 1973).

Calcium antagonists have been observed to antagonise the depressant effects of NA on cortical neurones. Lanthanum,

Verapamil and manganese antagonised depressant responses of cortical neurones to NA (Yarbrough et al, 1974). Similarly, ethanol, cocaine, procaine and lidocaine (Yarbrough et al, 1974) as well as neomycin and ruthenium red (Phillis, 1974) antagonised the inhibitory action of NA on cortical neurones.

A recent series of experiments have investigated the interaction between the tricyclic antidepressant drugs imipramine and desipramine, and the responses of single cortical neurones to NA (Bradshaw et al, 1971a; 1974). Both imipramine and desipramine can modify both excitatory and depressant responses to NA. A small dose of either antidepressant drug potentiated the response whereas a larger dose usually antagonised the response to NA (Bradshaw et al, 1974).

1:4.1.2. 5-hydroxytryptamine

The effects of 5HT on single neurones in the cerebral cortex were investigated by Krnjevic & Phillis (1963). These authors observed both excitatory and inhibitory responses to 5HT. As with NA, there was a predominance of depressant responses. Roberts & Straughan (1967) could also observe both excitatory and depressant responses of single cortical neurones to 5HT, but in contrast to Krnjevic & Phillis (1963), observed mainly excitatory responses. Johnson et al, (1969b) criticised the use of barbiturate anaesthesia by Krnjevic & Phillis (1963). Johnson et al (1969b) showed that, in barbiturate anaesthetised preparations, 5HT evoked largely depressant responses. In contrast, in encephale isolé or halothane

anaesthetised preparations, 5HT had a predominantly excitatory effect on single cortical neurones (Johnson et al, 1969b).

Later reports have demonstrated both excitatory and depressant responses to 5HT in the cerebral cortex of the cat (Bradshaw et al, 1971a; 1974; Bevan et al, 1974a) and rat (Stone, 1974).

Excitatory responses to 5HT could be reversibly antagonised by LSD, BOL, methysergide and cinanserin (Roberts & Straughan, 1967) whereas depressant responses to 5HT and excitatory responses to ACh were unaffected (Krnjevic & Phillis, 1963; Roberts & Straughan, 1967). Similarly, neither excitatory nor depressant responses to 5HT were affected by the adrenergic antagonists phentolamine and sotalol (Johnson et al, 1969a). However, Frederickson et al (1972) reported that depressant responses to 5HT could be antagonised by the adrenergic antagonists phentolamine, phenoxybenzamine and INPEA.

It has recently been reported that excitatory responses to 5HT can be potentiated by methysergide (Bevan et al, 1974b). Excitatory responses to 5HT were potentiated when methysergide was ejected with a low current, but the potentiation was superseded by antagonism when the intensity of the ejecting current was increased. Excitatory responses to 5HT were affected in a similar manner by sotalol: both potentiation and antagonism could be observed (Bevan et al, 1974b). Strychnine antagonised the depressant action of 5HT on neurones identified as pyramidal tract neurones (Stone, 1973a).

Lanthanum, verapamil and manganese antagonise the depressant effects of 5HT on cerebral cortical neurones (Yarbrough et al, 1974). Similarly, ethanol and the anaesthetic agents cocaine, procaine and lidocaine (Yarbrough et al, 1974) as well as neomycin and ruthenium red (Phillis, 1974) antagonised the inhibitory actions of 5HT on cerebral neurones. These diverse agents seem to share only the ability to interfere with calcium metabolism (Yarbrough et al, 1974).

The tricyclic antidepressant drugs imipramine and desipramine could modify both excitatory and depressant responses to 5HT (Bradshaw et al, 1971a; 1974). Small doses of imipramine or desipramine potentiated responses whereas larger doses antagonised responses to 5HT. Imipramine proved to be more potent than desipramine in modifying responses to 5HT, whereas the converse was true with respect to NA responses (Bradshaw, Roberts & Szabadi, 1973a).

1:4.1.3. Dopamine

DA was originally reported to cause a short latency depression of both spontaneous and amino-acid induced firing of cortical neurones (Krnjevic & Phillis, 1963). More recent reports have demonstrated excitatory responses of cortical neurones to DA. For example, Stone (1973b) reported both excitatory and depressant responses to DA in the cerebral cortex of the halothane and urethane anaesthetised rat.

Both excitatory and depressant responses of single cortical neurones to DA could be antagonised by chlorpromazine (Stone, 1974; Stone & Bailey, 1975).

1:4.1.4. Mescaline

Krnjevic and Phillis (1963) reported that the exclusive action of mescaline on single neurones in the cerebral cortex of the cat was one of depression. In contrast, Roberts and Straughan (1968) reported that mescaline could evoke both excitatory and depressant responses when applied to cortical neurones. This was confirmed later by Bradshaw, Roberts & Szabadi (1971b) who pointed out the high correlation between the effects of mescaline and NA. Furthermore, cross-desensitisation between the responses to mescaline and NA has been demonstrated, although it did not appear to be specific as responses to 5HT and ACh were often affected as well (Bevan et al, 1974a). The effect of mescaline on responses to NA and 5HT was also studied on cells which did not respond to mescaline (Bevan et al, 1974a). The simultaneous application of mescaline resulted in antagonism of excitatory responses of single cortical neurones to NA and 5HT.

Excitatory responses to mescaline could be antagonised and potentiated by sotalol and methysergide (Bevan et al, 1974b). When the antagonist was applied with a low ejecting current, the response to mescaline was potentiated. This potentiation was superseded by antagonism when the intensity of current ejecting the antagonist was increased. Responses to ACh were not affected. Depressant responses to mescaline were antagonised by methysergide, but not by sotalol, although the number of cells tested was small (Bevan et al, 1974b).

1:4.1.5. Acetylcholine

Krnjevic (1964) reported that the predominant effect of ACh on cortical neurones was one of excitation. Other reports however, have demonstrated depressant responses to ACh of cortical neurones in the cat (Spehlmann, 1963; Randic, Siminoff & Straughan, 1964; Crawford & Curtis, 1966) and in the rat (Stone, 1972). The response of a neurone to ACh was antagonised by intravenous injection of a small dose of barbiturate, urethane or chloralose (Crawford & Curtis, 1966).

Excitatory responses to ACh could be reversibly antagonised by atropine (Krnjevic & Phillis, 1961; Crawford & Curtis, 1966; Bevan, Bradshaw & Szabadi, 1975b) and hyoscine (Krnjevic & Phillis, 1962; Crawford & Curtis, 1966). Bevan et al (1975b) demonstrated that atropine shifted the current-response curve for ACh to the right in an approximately parallel fashion, indicating a competitive antagonism of ACh responses by atropine. A dose-ratio of 12 was obtained in these experiments. Clarke, Forrester and Straughan (1974) constructed cumulative dose-response curves on the basis of single applications of ACh. These curves were displaced to the right in an approximately parallel fashion in the presence of atropine. Stone (1972) reported that ACh could evoke excitatory, but not depressant, responses when applied to identified pyramidal tract neurones in the cortex. Non-pyramidal tract neurones were excited or depressed by ACh. Furthermore, atropine and hyoscine could antagonise both excitatory and depressant responses to ACh.

Excitatory responses to ACh were potentiated by the cholinesterase inhibitors neostigmine and edrophonium (Krnjevic & Phillis 1962).

Recently, the interaction between excitatory responses to ACh and the tricyclic antidepressants imipramine and desipramine has been investigated (Bevan, Bradshaw & Szabadi, 1973a; 1975a). Imipramine and desipramine could both potentiate and antagonise excitatory responses to ACh; lower doses potentiated whereas higher doses antagonised the responses. Excitatory responses to carbachol were affected in a similar way indicating that potentiation was not due to inhibition of cholinesterase activity. Atropine was also shown to have a dual effect on excitatory responses to ACh (Bevan et al, 1973c; 1975a). Similarly to imipramine and desipramine, low doses of atropine potentiated whereas higher doses antagonised the responses to ACh.

Neither tubocurarine nor dihydro-β-erythroidine was an effective antagonist of ACh excitations (Crawford & Curtis, 1966); however, ACh depressions could be antagonised by tubocurarine and dihydro-β-erythroidine as well as by atropine and hyoscine (Phillis & York, 1967). Depressant responses were also antagonised by strychnine (Jordan & Phillis, 1972), although these results were not confirmed (Stone, 1972). It has also been reported that the depressant responses of cortical neurones to ACh can be antagonised by nickel, ethanol, cocaine and procaine (Yarbrough et al, 1974).

1:4.2 CAUDATE NUCLEUS

1:4.2.1. Dopamine

In the caudate nucleus of the cat, McLennan and York (1967) reported that DA could both excite and depress neurones. Most cells were induced to fire by the application of excitant amino acids (L-glutamate or dl-homocysteic acid) and few spontaneously active neurones could be found.

Later Herz and Zieglgansberger (1968) reported that the only response to NA of single neurones in the caudate nucleus was that of depression. Woodruff, Elkhawad, Crossman and Walker (1974) also reported only depressant responses to DA. It is well documented, however, that DA can evoke both excitatory and depressant responses when applied to single neurones in the caudate nucleus. For example, Connor (1970) could observe excitatory and depressant responses to DA in the unanaesthetised cat, and similar findings have been reported in the urethane (Gonzalez-Vegas, 1974), Penthrane (Spencer & Havlicek, 1974) and halothane (Siggins, Hoffer & Ungerstedt, 1974) anaesthetised rat. Similarly, excitatory and depressant responses, as well as biphasic responses (consisting of an initial depression followed by an excitation) have been reported in the caudate nucleus of the squirrel monkey (York, 1972) and in the putamen of cats (York, 1970).

The α-adrenergic antagonist phenoxybenzamine, but not the β-adrenergic antagonist dichloroisopropyl noradrenaline, antagonised both excitatory and depressant responses to DA

(McLennan & York, 1967; York, 1967). Excitatory, depressant and biphasic responses to DA could also be antagonised by chlorpromazine (York, 1970; 1972) phentolamine and sotalol (York, 1970). It has also been reported that depressant responses to DA can be antagonised by papaverine, bulbocapnine (Gonzalez-Vegas, 1974) and ∝-methyldopamine (Connor, 1970).

The phosphodiesterase inhibitors aminophylline, and 1-methyl,3 iso-butyl xanthine potentiated the depressant effects of DA (Siggins et a l, 1974). In addition, these authors showed that, in contrast to Gonzalez-Vegas (1974) papaverine could also potentiate the depressant effects of DA (Siggins et al, 1974).

1;4.2.2. Noradrenaline

NA, when applied to single spontaneously active neurones in the caudate nucleus of the cat, evoked both excitatory and depressant responses (Salmoiraghi & Stefanis, 1965; Bloom, Costa & Salmoiraghi, 1965). In contrast Herz and Zieglgansberger (1968) reported exclusively depressant effects of NA on spontaneously active cells, and cells induced to fire by excitant amino acids in the caudate nucleus of the rabbit. Excitatory and depressant responses to NA have been reported in the putamen of the cat (York, 1970) and in the caudate nucleus of the rat (Spencer & Havlicek, 1974).

Excitatory responses to NA could be antagonised by the ∝-adrenergic antagonist phentolamine and depressant responses were antagonised by the β-adrenergic antagonists INPEA and sotalol (York, 1970).

1:4.2.3. 5-hydroxytryptamine

Both excitatory and depressant responses of single neurones to 5HT have been observed in the caudate nucleus of cats (Salmoiraghi & Stefanis, 1965) and in the putamen of cats (York, 1970). Excitatory responses to 5HT could be antagonised by phentolamine, whereas depressant responses were antagonised by sotalol.

1:4.2.4. Acetylcholine

In the unanaesthetised decerebrate cat, ACh evoked both excitatory and depressant responses when applied to spontaneously active neurones in the head of the caudate nucleus (Bloom et al, 1974). Cells induced to fire by the application of glutamate were invariably depressed by ACh. In agreement with Bloom et al (1974) Salmoiraghi and Stefanis (1965) and McLennan and York (1966) could observe both excitatory and depressant responses to ACh in unanaesthetised encephale isolé preparations. Atropine, but not hexamethonium, was able to antagonise both excitatory and depressant responses to ACh (McLennan & York, 1966; York, 1970). Later, however, York (1970) reported that depressant responses to ACh could be antagonised by hexamethonium and excitatory responses could also be antagonised by curare.

CHAPTER 2

METHODS

MICROELECTROPHORETIC EXPERIMENTS

2:1 MICROPIPETTES

The technique of microelectrophoresis involves the use of small electrical currents to expel minute quantities of drugs from fine micropipettes into the tissue (Curtis, 1964).

2:1.1 MANUFACTURE OF MICROPIPETTES

Micropipettes were manufactured from Pyrex glass capillary tubing (1.5 mm diameter, 1.00 mm bore) using a modification of the method described by Herz, Wickelmaier and Naciemiento (1965). The glass was cut to a uniform length (approximately 100 mm). Six pieces were bundled together and smeared with "Araldite" (CIBA) at each end. A brass ring was then placed over each end of the tubes and the completed assembly, or blank, heated in an oven ($100^{o}C$; 30 min). The six glass capillary tubes were thus securely bound to each other, and to the brass rings, by the adhesive. To ensure that the blank was clean (see 2:1.2), the blank was washed in chromic acid, followed by two distilled water rinses and oven drying.

Two multibarrelled micropipettes were made from each blank using a locally made machine (see Fig 1). The brass rings at the end of each blank were securely clamped in $\frac{1}{4}$" chucks so that the blank was held vertically with the heating coil around the centre of the glass (Fig 1). The heating coil was made from Nichrome wire and heated by a current from a 6V DC source. The glass at the centre of the blank was softened by switching on the heating coil, and, whilst keeping the top chuck stationary, the bottom chuck was turned through 180^{o} and the heating coil

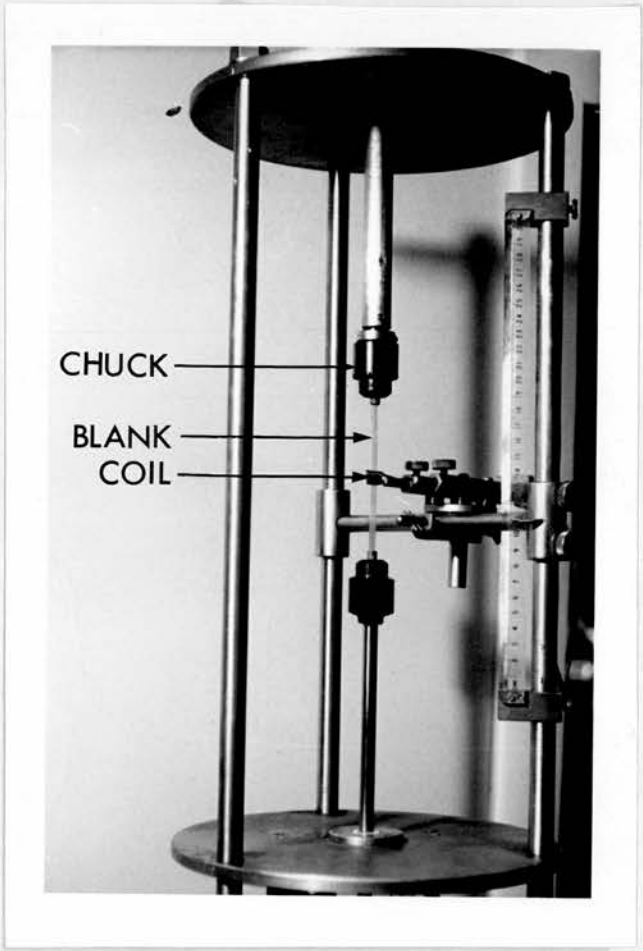


FIG. 1. Photograph of the Puller used to manufacture the micropipettes used in these experiments. See text (2:1.1) for details.

switched off. This procedure fused the individual glass pipettes of the blank together so that the blank was secure at the centre as well as at either end. After the glass had cooled, the heating coil was repositioned at the centre of the twisted glass and switched on.

As the glass softened, the bottom chuck pulled the two brass rings apart drawing the glass capillaries out until they separated. Thus each chuck held a six-barrelled glass micropipette. The overall tip diameter of micropipettes produced in this way was less than 1 µm.

2:1.2 FILLING OF MICROPIPETTES

The individual capillaries, or barrels, of a micropipette were filled with aqueous solutions of the drugs whose effects were to be studied. Two barrels were always filled with 4M NaCl solutions for the recording of extracellular action potentials (see 2:3.1) and for current balancing (see 2:3.2.4).

Great care was taken to ensure that everything used for manufacturing micropipettes was clean and dust free, as the tips of micropipettes can be easily blocked or obstructed. Thus the glass used for manufacturing micropipettes was thoroughly washed with chromic acid, and drug solutions used for filling micropipettes were centrifuged (1500 g; 30 min) immediately before use to sediment any dust particles.

Two methods of filling micropipettes were used.

2:1.2.1. Diffusion

This method, described by Curtis (1964), involved two processes. First, the air in the barrels of the

micropipette had to be displaced with distilled water, and second, the water in each barrel of the micropipette had to be replaced with the appropriate drug solution.

The micropipette, secured so as to protect the delicate tip, was immersed in a beaker of double glass distilled water at 80°C. The beaker, in turn, was placed in a sealed chamber from which the air was then evacuated so that the water in the beaker boiled gently for about 20 min. This forced the air out of the micropipette barrels, including the air in the narrow bore of the very tip of the barrels, and replaced the air with distilled water. The water was then removed from each barrel of the micropipette down to the level of the shank using a thin Portex tube and a syringe. The barrel was then filled with a solution of the appropriate drug using a thin Portex tube. Care was taken not to introduce air bubbles into the barrels during this process. After filling, the micropipettes were stored for 36 hr before use to allow the drug molecules to diffuse into the tips of the barrels. To inhibit oxidation of drugs during this time, micropipettes were stored at 4°C in a nitrogen atmosphere.

Immediately before use, the tip of a micropipette was broken under microscopic control by bumping the tip against a glass rod. The overall diameter of the micropipette tip after breaking varied between 3 and 6 μm; this gave a DC resistance of the recording barrel of between 2 and 5 megohms, when measured with the tip immersed in 0.9% NaCl solution.

An advantage of this technique of micropipette filling is that the micropipettes can be prepared in advance and in sufficient numbers to serve several experiments. Thus experiments can proceed as soon as the experimental animal has been prepared. However, the necessity of storing filled micropipettes to allow diffusion to occur would be a disadvantage if drugs of an unstable nature were involved.

2:1.2.2. Centrifugation

In this method, described by Curtis (1964), drug solutions were forced into the tips of the micropipette by using high centrifugal forces.

First the tips of the empty micropipettes were broken under microscopic control to give an overall tip diameter of about 4 μm (see above). Using thin Portex tubes, each barrel of the micropipette was then filled with the appropriate drug solution and the micropipette clamped into a centrifuge tube. The micropipette was then centrifuged for 30 min at 1500 g. The micropipettes were then ready for use.

As the micropipette tip was broken before being filled with solutions, some of the drug solution could leak from micropipettes filled by centrifugation. Micropipettes filled in this way were thus not suitable for storage; micropipettes would have to be prepared fresh for each experiment.

2:2 PREPARATION OF EXPERIMENTAL ANIMAL

The microelectrophoresis experiments described in this thesis were performed on rats. Many of the experiments involved the study of single neurones in the caudate nucleus where rats offered several advantages over other experimental animals eg cats. The caudate nucleus in rats lies approximately 2.5 mm below the surface of the brain and can thus easily be reached by introducing a 6-barrelled micropipette through the cortex. Thus there is no surgical intervention on the brain and no brain tissue has to be removed eg. the overlying cerebral cortex (see McLennan & York, 1966). Thus the physiological integrity of the brain was preserved as much as possible.

Experiments were performed on male albino Wistar rats weighing between 250 and 300 g.

2:2.1 ANAESTHESIA

Halothane anaesthesia was routinely used in all experiments described in this thesis.

The choice of anaesthetic is crucial in microelectrophoresis experiments as some anaesthetics can alter the sensitivity of neurones to putative neurotransmitter agents (Curtis, 1964). It has been shown, for example, that, compared to unanaesthetised preparations, barbiturate anaesthesia significantly reduced the proportions of excitatory responses of single neurones to ACh in the cerebral cortex, caudate nucleus (Bloom et al, 1965) and brain stem (Bradley & Dray, 1973). Similarly, in barbiturate anaesthetised preparations, excitatory responses to monoamines of single neurones in the cerebral cortex (Johnson et al,

1969b) and caudate nucleus (Spencer & Havlicek, 1974) are almost absent. Using halothane anaesthesia however, both excitatory and inhibitory responses of single neurones to monoamines (Roberts & Straughan, 1967; Johnson et al, 1969b) and to ACh (Crawford, 1970) may be observed. Furthermore, whereas pentobarbitone reduces the glutamate sensitivity of neurones, halothane (1%) has no effect (Richards & Smaje, 1974). Moreover, being a volatile anaesthetic, administration of halothane and maintenance of anaesthesia are easily achieved.

Anaesthesia was induced with halothane (Fluothane ICI) delivered in oxygen from a temperature and oxygen flow-rate compensated vapouriser (Fluotec Mk 3, Cyprane Limited). A tracheostomy was then performed and a tracheal cannula inserted. Animals respired spontaneously throughout the experiment.

The cannula used consisted of a Pyrex glass tip, shaped to fit easily into the trachea, to which two Portex tubes were attached via a Y piece. The oxygen/halothane mixture was introduced into one Portex tube and flowed out of the other. The flow rate used was 500 ml/min. During early experiments, many animals were lost due to the cannula orifice becoming blocked with dried blood and mucus. This was overcome by regularly siliconising (Repelcote, Hopkins & Williams Ltd) the cannula.

During the course of the surgery, the halothane concentration was reduced so that, when all the surgery had been completed, the halothane concentration was 0.5-1%; it was maintained at this level throughout the experiment.

2:2.2 PHYSIOLOGICAL MONITORING

Several physiological parameters were continuously monitored during the course of an experiment.

2:2.2.1. Rectal temperature

The rectal temperature was measured on a thermometer or via a thermosensitive rectal probe connected to a galvanometer (Ellab Ltd). The rectal temperature was maintained between 37° and 38°C throughout the experiment by a heating pad which was placed under the belly of the animal. The heating pad was controlled by a thermosensitive probe which was placed between the pad and the animal.

2:2.2.2. Electrocardiogram

The electrocardiogram (ECG) was continuously monitored throughout the experiment. A stainless steel electrode was inserted beneath the skin of the animals' forelimbs. The electrodes used were hypodermic needles, (19G1 gauge, Gillette). The electrodes were connected via a wide band AC preamplifier (Grass 7P 3A) to a driver amplifier (Grass 7DA). Both the preamplifier and the animal were earthed, providing the third pole necessary for ECG recording. The ECG was written on paper using a Grass model 7 polygraph.

2:2.2.3. Electrocorticogram

The electrocorticogram (ECoG) was continuously monitored from the side of the brain contralateral to that used for recording action potentials. Two small holes were made in the skull using a dental burr (Ash, round 1 size).

A silver ball electrode was then carefully placed on the surface of the dura in each hole and cemented in place using dental acrylic (Simplex, Dental Fillings Ltd). The electrodes were connected via a wide band AC EEG pre-amplifier (Grass 7P5A) to a driver amplifier (Grass 7DA) and the ECoG written out by one pen of a Grass model 7 polygraph.

2:2.3 PREPARATION OF RECORDING SITE

The animal's head was held in a stereotaxic frame and the skull exposed. An area of the brain was exposed for the recording of action potentials according to the method described by Bradshaw and Szabadi (1972).

2:2.3.1. Cerebral Cortex

In order to expose an area of the cerebral cortex for recording, a small hole was drilled in the skull using a small dental burr (Ash, Round 1 size). An incision was then made in the dura under microscopic control. A micropipette could then be introduced through the incision and into the brain tissue under microscopic control.

2:2.3.2. Caudate nucleus

An area of the cerebral cortex was exposed as described above. The stereotaxic coordinates chosen for this exposure were A 8.0; L 2.4 (Konig & Klippel, 1963). At these coordinates the caudate nucleus lies 2.5 - 3.00 mm below the surface of the brain (Konig & Klippel, 1963). Thus, under microscopic control a micropipette could be lowered through the cortex to the top of the caudate nucleus.

In practice, then, studies could be performed in both the cerebral cortex and caudate nucleus using the same micropipette and the same micropipette track.

2:2.4 LOCATION OF SPONTANEOUSLY ACTIVE NEURONES

The method of preparing the recording site described above provided a stable recording situation free from respiratory and circulatory pulsations. To maintain this stability, micropipettes were advanced through the tissue slowly. To achieve a slow but steady progress through the tissue, the micropipette was advanced by means of a microdrive (La Precision Cinematographique) driven by a 3V DC motor; the motor could be controlled either manually, or by an automatic device specially built for this purpose (see paragraph below).

This micropipette advancing device was actually based on the circuit employed in the sequential timing device used to control the electrophoretic currents (2:3.2.2). Two timers were employed which operated alternatively in a continuous cycle. The time period for the first timer was fixed at 250 msec, whilst the second timer was variable from 4 to 15 seconds. The microdrive motor was driven during the period of operation of the first timer by a variable current so that the micropipette could be advanced in steps of 2 - 20 µm. Thus, the device could advance the micropipette through the tissue in steps varying from 8 µm/min to 300 µm/min. The device could be instantly stopped once the micropipette was in the vicinity of a spontaneously active neurone.

Spontaneously active single neurones selected for study

were described as those with a stable baseline firing rate, and whose spikes were of unchanging amplitude above 300 μV. Using the techniques described above, such neurones could be routinely studied for periods of up to 5 hours.

2:2.5 HISTOLOGY

Histological examination was performed on rat brains which had been used for studies in the caudate nucleus.

First, the multibarrelled micropipette was removed from the brain. This allowed the track left by the micropipette to fill with blood which facilitated subsequent detection. The animal was sacrificed by an intraperitoneal injection of a lethal dose of pentobarbitone sodium (Nembutal, Abbott Laboratories), the brain quickly removed and immersed in a solution of 10% formaldehyde in 0.9% NaCl. After fixation (7 - 14 days) the hind-brain was removed and the fore-brain mounted on a microtome. The tissue was frozen using carbon dioxide gas (Distillers Co) and 20 μm sections cut. For staining, the sections were mounted on glass microscope slides which had been coated with gelatine (2.5% gelatine with 1% phenol in distilled water). Slides were stored under formalin vapour until staining could begin.

The slides were first washed in distilled water before being immersed in the staining solution for 15 min at room temperature. The staining solution was a 1% aqueous solution of Toluidin Blue (Gurr). After staining, the slides were rinsed in distilled water followed by rinsing in 95% alcohol. The slides were then immersed in

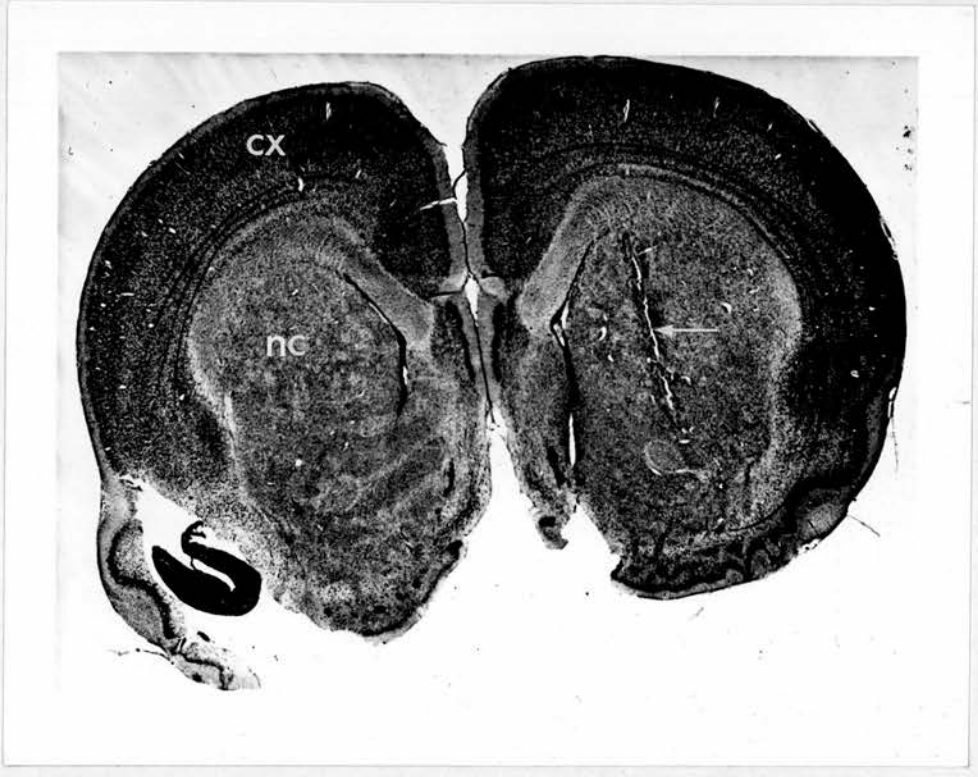


FIG. 2. Photograph of a transverse section of the rat brain.

CX : Cerebral cortex; NC : Nucleus caudatus putamen.

Approximate Stereotaxic coordinate A8.0 (Konig & Klippel, 1963).

The arrow in the left hemisphere indicates the lesion made by the micropipette. See text (2:2.5) for details of histological preparation.

absolute alcohol (for differentiation) followed by xylene. Finally, each section was covered by a drop of Depex mounting material (Gurr) and a cover-slip positioned. The correct location of the micropipette track could be verified, using a 10X magnifying glass, by reference to the atlas (Konig & Klippel, 1963).

Figure 2 shows an example of a section of rat brain stained using this technique.

2:3 RECORDING AND DRUG APPLICATION

Conventional techniques were used to record extracellular action potentials and to apply drugs into the local environment of a single spontaneously active neurone. A schematic diagram of the circuits is shown in Figure 3.

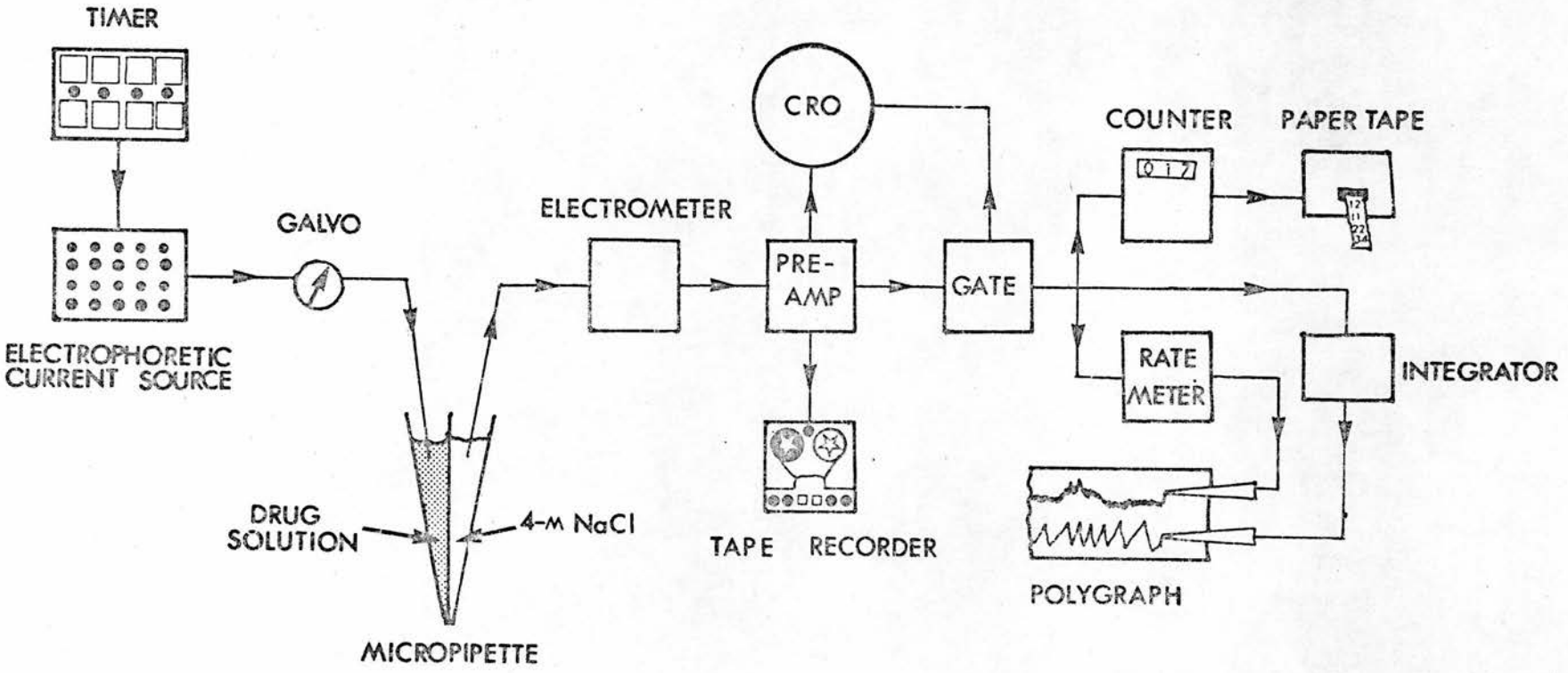
2:3.1 RECORDING OF ACTION POTENTIALS

Extracellular action potentials were recorded through one barrel of the multibarrelled micropipette as described by Roberts and Straughan (1967).

The animal was electrically earthed via the stereotaxic frame. The recording barrel of the micropipette contained 4M NaCl, was connected to a capacitance neutralising electrometer (Bioelectric NF-1, gain 2X). The output of the electrometer was fed to a preamplifier (Tektronix FM122, frequency range 0.08 - 10 kHz, gain 100X), and then to a cathod ray oscilloscope (Tektronix 565, final gain 200X). This signal could also be recorded on magnetic tape for later analysis (Ferrograph Series Seven). Z-axis modulation was used to intensify the fast transients on the oscilloscope screen (Pokrovsky, 1960).

Action potentials of equal amplitude were separated from the electrical noise, and from action potentials of lower amplitude by an electronic gating device. The output from the gate was simultaneously displayed on the oscilloscope screen above the original signal so that the action potentials passing through the gate were easily distinguishable. Three methods of recording the digital

FIG. 3. Schematic diagram of the circuits used in microelectrophoresis experiments.



output of the gate were used.

First, the signal was fed to a special counter (Venner Electronics, type 029/X1) which displayed the total number of action potentials counted in a given epoch. This information could be printed on to paper tape (Kienzle printer).

Second, the signal was fed to a unit integrator (Grass, UI-1) which integrated the number of action potentials passing through the gate. The output of the integrator was fed to a driver amplifier (Grass model 7DA). Thus the integrated signal was described by the pen-writing oscillograph (Grass model 7). The system was calibrated so that the integrator reset to zero when the oscillograph pen reached full-scale deflection. The resulting trace is shown in Figure 4. Thus, each excursion of the pen corresponded to the number of action potentials produced in a given time.

Third, the signal was fed to a rate-meter (Ecko, model N 522 C). The output of the rate-meter, the number of action potentials produced per unit time (the firing rate of the neurone), was fed via a low-level DC preamplifier (Grass 7P1A) to a driver amplifier (Grass model 7DA). Thus the firing rate was written by a pen-writing oscillograph. The integrator and rate-meter traces were written out simultaneously one above the other for easy comparison (see Figures 3 and 4).

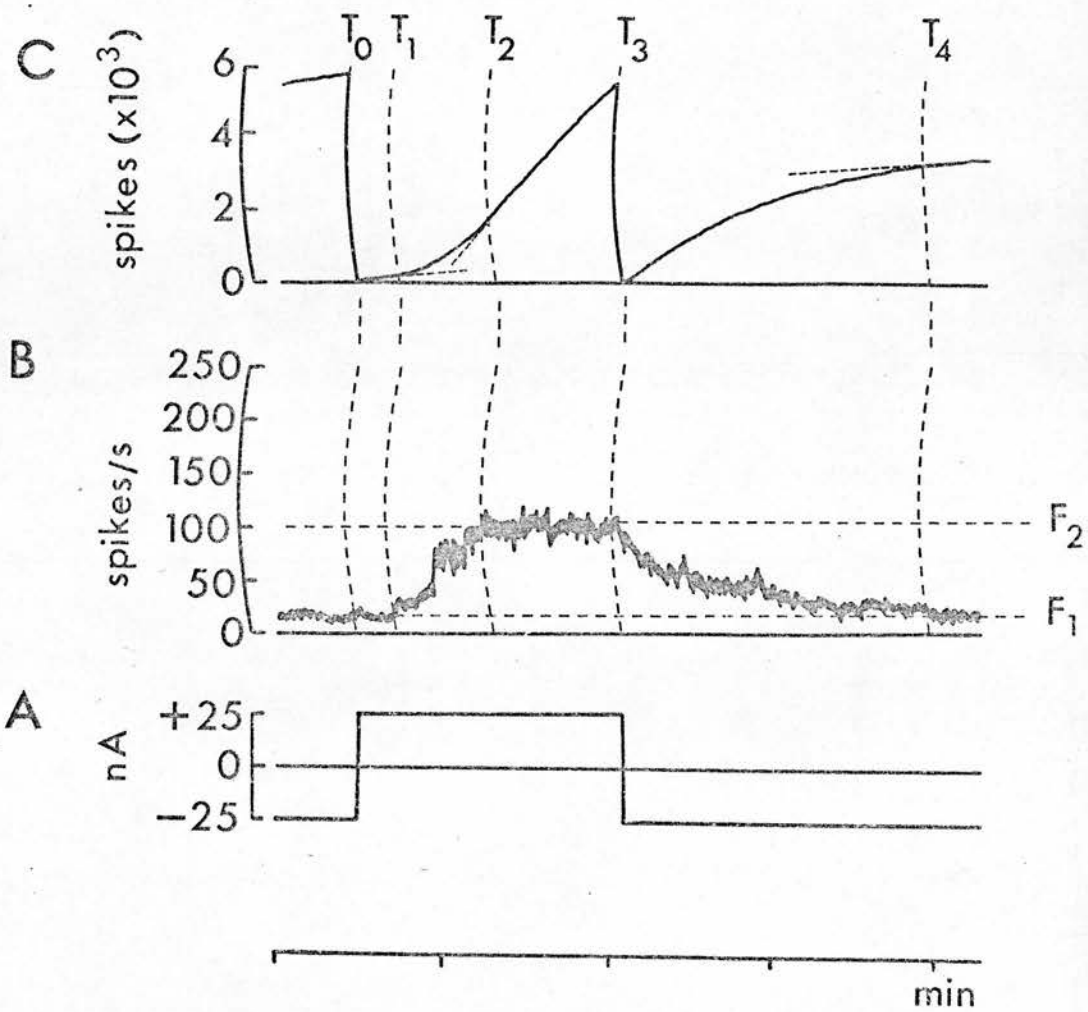


FIG. 4. Parameters of an excitatory response of a single cortical neurone to acetylcholine.

(A) Electrophoretic current passed through the solution of acetylcholine chloride contained in one barrel of the micropipette (ordinate: current intensity (nA); abscissa: time (minutes).

(B) Ratemeter recording of the firing rate (ordinate: firing rate (spikes/s); abscissa: time (minutes)).

(C) Cumulative record of the total number of action potentials generated by the neurone (ordinate: total spikes; abscissa: time (minutes)).

Response parameters (see 2:4.1) are indicated by the letters above and to the right of the traces.

(After Bradshaw et al, 1973c).

2:3.2 APPLICATION OF DRUGS

All drugs were applied as ions from aqueous solutions by microelectrophoresis.

The drug solutions used in the experiments were:

1. Dopamine hydrochloride (0.1 or 0.2M, pH 4.0-4.5; Koch-Light).
2. (-)-noradrenaline bitartrate (0.1 or 0.2M, pH 3.0-3.5; Koch-Light).
3. 5-hydroxytryptamine bimaleate (0.1 or 0.2M, pH 3.5; Koch-Light).
4. Mescaline hydrochloride (0.05M, pH 3.5-4.5; Sigma).
5. Acetylcholine chloride (0.1 or 0.2M, pH 3.6; Koch-Light).
6. Sodium (-)-glutamate (0.05M, pH adjusted to 8.5 by the addition of 0.1N NaOH; British Drug Houses).
7. Desipramine hydrochloride (0.15M, pH 4.5; Geigy).
8. Iprindole hydrochloride (0.05M, pH 4.5; John Wyeth and Brother).

2:3.2.1. Electrophoretic Current Source

Drug ejecting and drug retaining currents (see 2:3.2.3 below) were provided by the circuit described in Figure 5. A 300V DC supply was reduced stepwise to obtain voltages, and thus currents, of an appropriate magnitude. The final output resistance of the circuit was 1000 megohmn which effectively minimised any changes in the current flow due to changes in the electrical resistance of the drug barrels (Roberts & Straughan, 1967). The current flow to each barrel of the micropipette could be measured on a Pye Scalamp galvanometer. Artefacts,

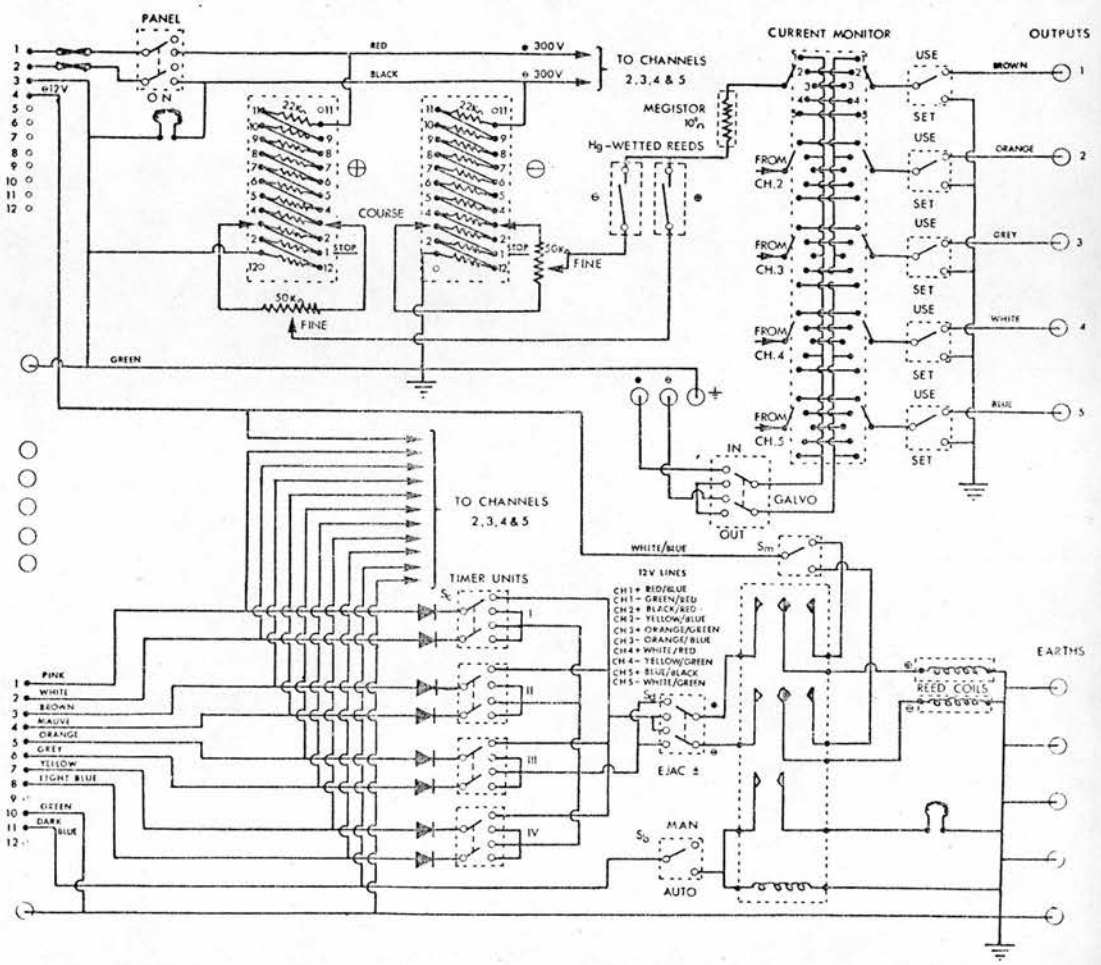


FIG. 5. Circuit diagram of the electrophoretic current source employed in these experiments.

See text (2:3.2.1) for explanation.

produced by switching from positive to negative current, for example, were minimised by the use of mercury-wetted reed switches. These were controlled by an independent 12V circuit. The reed switches could be operated manually (switch Sm in Figure 7) or automatically by a sequential timing device (Bevan & Bradshaw, 1973). The current source provided for up to 5 independent electrophoresis channels.

2:3.2.2. Sequential timers

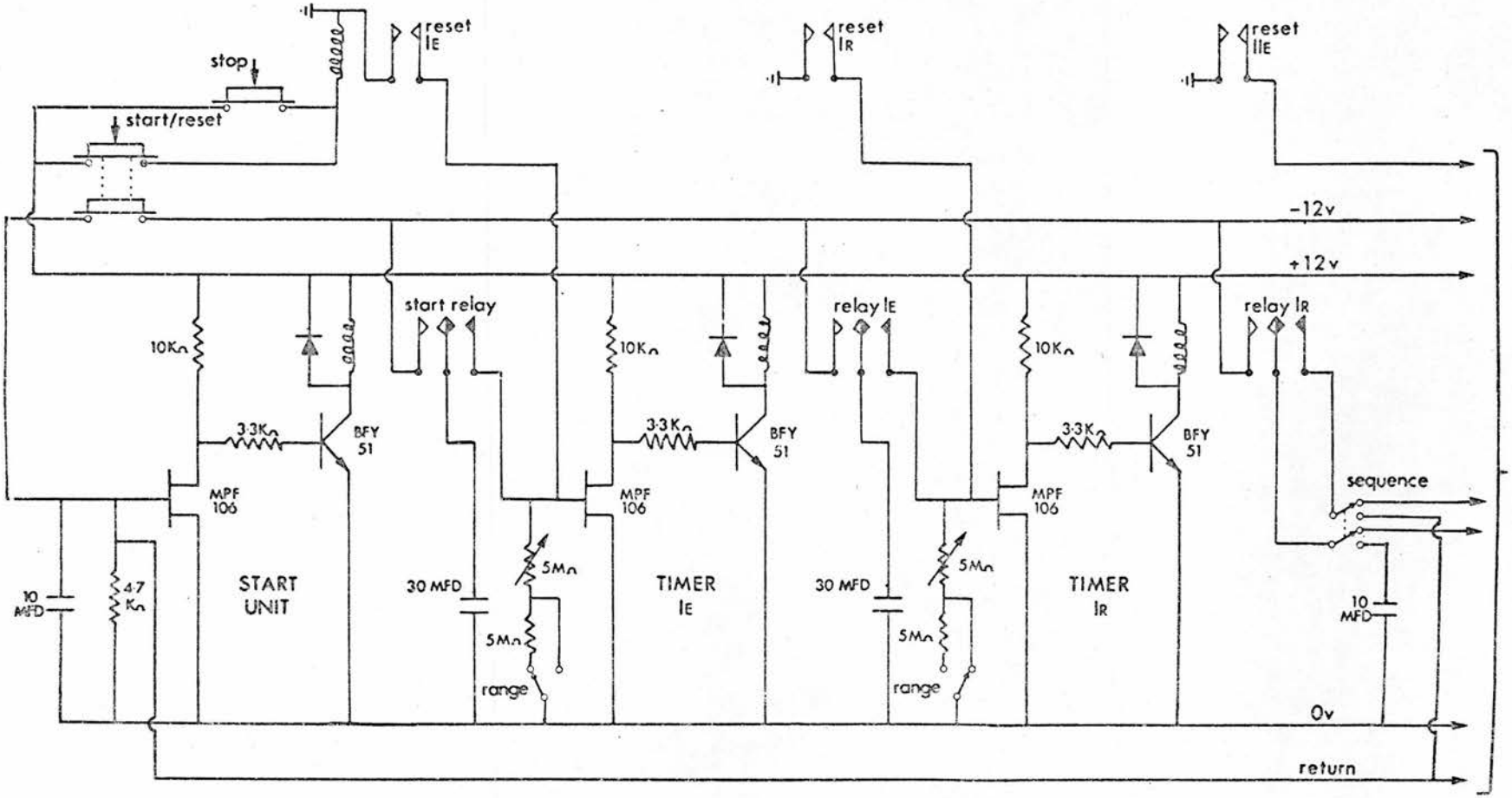
In the drug interaction studies described below the change in the size of a neuronal response to an agonist was followed in time. It was important to ensure that the observed change in the size of a response was not due to any physical factors, but was an effect of a drug interaction. Physical factors which can alter the size of a response include the parameters of drug ejection and retention (Bradshaw, Roberts & Szabadi, 1973b). Thus, during a drug interaction study, the ejection and retention times, as well as the ejection and retention currents, must be kept constant.

A sequential timing device was built which ensured that the ejecting and retaining currents were applied to the drug barrels in a regular sequence during a study so that each ejection period resulted in a standard amount of drug released from the barrel (Bradshaw, Szabadi & Roberts, 1973c).

The sequential timing device consisted of eight individual timers, and the circuit is shown in Figure 6.

The timers were linked in pairs to form four timer

FIG. 6. Circuit diagram of the sequential timing device used in these experiments. The start unit (used to initiate the timing sequence) and one Timer Unit only is shown. See text (2:3.2.2) for details.

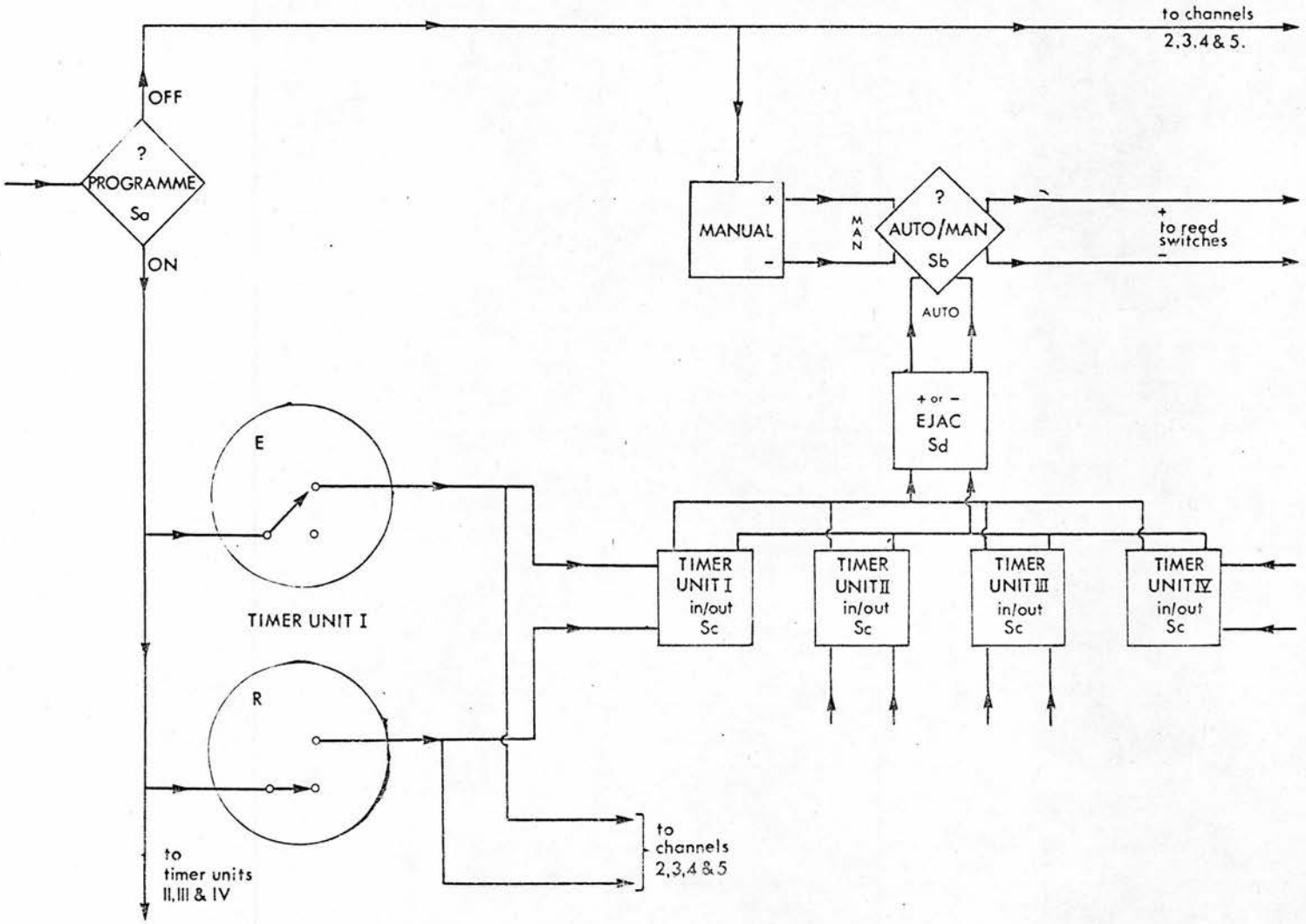

stop
start/reset
reset IE
reset IR
reset IIE
-12v
+12v
start relay
relay IE
relay IR
10KΩ
3·3KΩ
BFY 51
MPF 106
10 MFD
4·7 KΩ
START UNIT
30 MFD
5MΩ
range
TIMER IE
TIMER IR
sequence
0v
return
to timer units II, III & IV

units (1, 11, 111 & IV). Of each unit, one timer controlled the ejection period (E timer) and the other the retention period (R timer), independent of the polarity of the current required for drug ejection (see 2:3.2.3). The timers operated sequentially (E followed by R; unit 1 followed by unit 11), and cyclically (unit IV followed by unit 1). The cycle could include one or all of the timers so that a sequence of up to eight independent times could be established and repeated indefinitely. The circuit enabled the programmed application of ejection and retaining currents from up to 4 electrophoresis channels in a regular cycle.

The timers were driven by a 12V DC supply and operated the mercury-wetted reed switches in the electrophoretic current source (see 2:3.2.1). More than one electrophoresis channel could be operated simultaneously by each unit so that current balancing (2:3.2.4) could be achieved throughout the experiment.

The programme was initiated by means of the switch Sa (Figure 7). The inclusion of each channel into the programme was determined by the switches Sb. Each channel could be operated by any, or all, of the timer units as determined by the switches Sc. If any channel was not controlled by a particular timer unit, the retaining current was automatically maintained while that timer unit was in operation. The direction of the current during the ejection period was controlled by the switches Sd.

FIG. 7. Schematic diagram of the circuits used in these experiments to programme the sequential application of electrophoretic voltages to the micropipette barrels. See text (2:3.2.2) for details.


to channels
2,3,4 & 5.
OFF
?
PROGRAMME
Sa
ON
MANUAL
+
−
MAN
?
AUTO/MAN
Sb
+
to reed
switches
−
AUTO
+ or −
EJAC
Sd
E
TIMER UNIT I
R
TIMER
UNIT I
in/out
Sc
TIMER
UNIT II
in/out
Sc
TIMER
UNIT III
in/out
Sc
TIMER
UNIT IV
in/out
Sc
to
channels
2,3,4 & 5
to
timer units
II, III & IV

2:3.2.3. Ejecting and Retaining Currents

Drug ions were ejected from barrels of micropipettes by a current passing through the drug solution in the barrel. Both positive and negative currents could be passed through each barrel of the micropipette. The animal was at ground (zero) potential (see 2:3.1). Thus positive current flowed through the barrel when the animal was the cathode, whereas, negative current flowed when the animal was the anode. The polarity of the current used to eject the drug ion depended on the charge of the drug ions in solution. Thus, glutamate, which is negatively charged at pH 8.5, was ejected from the micropipette by a negative current. All other drugs used were expelled from the micropipette as positive ions by a positive current passing through the appropriate barrel. In between drug applications a retaining current flowed through the drug solutions to prevent the diffusion of drug ions from the barrels. The retaining current for each drug was opposite in polarity to the current which ejected the drugs from the micropipette (i.e. positive current for glutamate, negative for all other drugs).

2:3.2.4. Current Balancing

To control for possible current effects, current balancing (Roberts & Straughan, 1967) was routinely used. The object of the current balancing procedure was to maintain a current flow through the micropipette as a whole which did not change in magnitude or polarity during drug ejection or retention periods. This was achieved by currents of appropriate polarity flowing through a 4M NaCl containing barrel of the micropipette (not the recording

barrel) so that the total positive and negative currents passed remained constant during the ejection and retention periods. For example, if a current of + 50 nA passed through a barrel to eject a drug, whereas a current of - 25 nA retained that drug in the barrel, then, during drug ejection periods a current of - 25 nA passed through the NaCl containing barrel, whereas, during drug retention periods, a current of + 50 nA passed through the NaCl containing barrel. Thus, although the direction and magnitude of the current flow changed in the drug barrel during drug ejection, the net change in the micropipette as a whole was always zero.

Drug ejection involved, then, the simultaneous changing of the polarity and magnitude of the current flow in two barrels of the micropipette. This could be achieved by using the sequential timing device described above (2:3.2.2).

2:4 ANALYSIS OF RESPONSES

In these experiments, action potentials generated by a single neurone were defined as spikes of equal amplitude which were clearly separated from the electrical noise level and from spikes of lower amplitude. Pharmacological responses were evaluated on the basis of the rate-meter and integrator (see 2:3.1) records.

2:4.1 RESPONSE PARAMETERS

The following parameters describe the response of a single neurone to a microelectrophoretically applied drug (see Figure 4, and Bradshaw et al, 1973c):

(a) The spontaneous (baseline) firing rate (F1)

(b) The response latency (latency of onset of the response, measured from the onset of the ejecting pulse) (T1)

(c) The maximum firing rate (the maximum firing rate achieved by the cell during the response) (F2-F1)

(d) The time taken to achieve the maximum firing rate (T2)

(e) The recovery time (the time taken for the recovery of the spontaneous firing rate after the termination of the ejecting pulse) (T4-T3)

(f) The total spike number.

2:4.2 CALCULATION OF TOTAL SPIKE NUMBER

The total spike number is a parameter which estimates the overall size of a response, and was thus used to assess the degrees of antagonism and potentiation in drug interaction studies (see 2:5). It was calculated by taking the difference between the number of action potentials produced during the response and subtracting the number of action potentials produced during an equivalent period when no drug was applied (see Bevan et al, 1973a). Thus, in the case of excitatory responses, the total spike number is the number of action potentials generated in response to the drug application, whereas, in the case of depressant responses, it is the number of action potentials whose generation was suppressed by the drug application.

Three methods of calculating the total spike number were developed.

2:4.2.1. Integration

This method required continuous on-line monitoring of the number of action potentials produced by the neurone. Impulses from an electronic gating device (see 2:3.1) were integrated and the output described by a pen-writing oscillograph (see 2:3.1). The total distance moved by the pen was proportional to the number of action potentials passing through the gate. The integrator was reset to zero at the beginning of every drug ejection period, whereas, the end of the response could be detected by a change in the slope of the integrator pen write-out (see Figure 4). Thus, comparison of the

number of excursions of the integrator pen before and during a response enabled the total spike number to be calculated.

2:4.2.2. Estimation

Another way to calculate the total spike number was to measure the area described by the response curve; that is, the area between the limits F2 and F1, and T1 to T4 in Figure 4. This area is directly proportional to the total spike number. Two methods of estimating the area of the response were used.

2:4.2.2.1. Tracing

The outline of each individual response was copied on to tracing paper and cut out. The size of each response was thus expressed as the weight of the response cut-out.

2:4.2.2.2. Planimetry

The area of the respons e was measured direct from the rate-meter record. A compensating polar planimeter (A. Ott, model 30.000.113) was used for this purpose.

2:5 DRUG INTERACTION EXPERIMENTS

The technique of microelectrophoresis was used to assess the interaction between tricyclic antidepressant drugs and putative neurotransmitters.

2:5.1 METHOD OF STUDY

The agonist (a putative neurotransmitter) was applied in a regular cycle. The intervals between agonist applications, and the durations of agonist applications, were kept constant using a sequential timing device (see 2:3.2.2). Similarly, during a study, the ejecting and retaining currents were kept constant. This ensured that the standard ejecting current pulses gave rise to standard pulses of agonist ejection (Bradshaw et al, 1973c). Several control responses were obtained before a drug-interaction study. The size of a response was expressed as the total spike number. Cells were excluded from drug-interaction studies if the variation in the size of the responses to the agonist exceeded $\pm$ 10% (see Bradshaw et al, 1974).

2:5.2 APPLICATION OF ANTIDEPRESSANTS

The tricyclic antidepressants were applied either continuously or in the form of a brief pulse.

2:5.2.1. Continuous Application

First, control responses to an agonist were obtained, as described above (2:5.1). Then, the antidepressant was ejected from the micropipette continuously, using low ejection currents (0-15 nA). The application of the antidepressant was continued even during the agonist applications. With this method the concentration of the

antidepressant would rise gradually to a plateau, and this concentration would then be maintained for the rest of the antidepressant application (Szabadi & Bradshaw, 1974). Once the application had ceased, the concentration would then gradually decline to zero.

This method was only successful on one occasion (see Figure 27). Almost invariably, during the application of the antidepressant, the spike height would be drastically reduced so that the study had to be abandoned. Usually, after terminating the antidepressant application, the spike height would recover. This effect probably reflects the local anaesthetic action of these drugs (Domenjoz & Theobald, 1959). This method was discontinued in favour of pulse application.

2:5.2.2. Pulse Application

First, control responses to an agonist were obtained, as described above (2:5.1). Then, the antidepressant was ejected from the micropipette as a brief pulse, the current through the antidepressant barrel being 30 - 100 nA passed for 30 - 80 seconds. This procedure did not interrupt the regular sequence of agonist application.

The difficulties associated with the continuous application of antidepressants were overcome using this technique. However, the higher currents which were used with pulse application revealed an agonistic action of the antidepressants (see Results). Little evidence of the local anaesthetic action was observed with this technique. Cells showing a reduction in spike height due to the application of an antidepressant were excluded from drug interaction studies.

2:5.3 CONTROL AGONIST

In any drug interaction regime it is necessary to establish the degree of specificity of the observed effects. This was accomplished in the experiments reported here by the use of a control agonist. Originally, in the study of the effects of tricyclic antidepressants on neuronal responses to monoamines, ACh was used as a control agonist. However, these experiments showed that responses of single neurones to ACh were affected in a similar manner to responses to the monoamines (Bradshaw et al, 1971a; Bevan et al, 1973c; 1975a). Therefore, in the experiments reported here, glutamate was used as the control agonist. Responses of single neurones to glutamate are not affected by tricyclic antidepressants (Bradshaw et al, 1974; Bevan et al, 1975a).

Ideally, the effect of the antidepressant should be studied on the test agonist and control agonist at the same time. However, the experiments reported here involved following the time-course of the changes in the size of the response to the agonist after the application of a brief pulse of the tricyclic antidepressant drug. This dictated that only one agonist could be tested at a time, as the inclusion of another (control) agonist into the sequence would necessarily increase the time between the applications of the test agonist. This usually prohibited the simultaneous examination of the effects of antidepressants on the responses to the control and test agonists. However, the interaction between antidepressants and the control and test agonists could still be tested on the same cell.

Once a successful drug interaction study had been completed with a test agonist, the study could be repeated on the same cell with the control agonist, using the same dose of tricyclic antidepressant drug.

2:5.4 DEFINITION OF EFFECTS

In the drug interaction studies described below, three possible effects could be observed.

2:5.4.1. Potentiation

A response was regarded as potentiated if there was an increase of at least 20% in the size of the response when compared to the size of the mean of the control responses, as determined by the total spike number (see 2:4.2).

2:5.4.2. Antagonism

A response was regarded as antagonised if there was a decrease of at least 20% in the size of the response when compared to the size of the mean of the control responses, as determined by the total spike number (see 2:4.2).

2:5.4.3. Reversal

Following the application of an antidepressant, the direction of a response (excitation or depression) was reversed, later to recover. For example, the cell first responded with a clear excitation to the agonist; following the application of the antidepressant, however, the same agonist evoked an opposite response (depression). Later, the original excitatory response to the agonist recovered.

BIOCHEMICAL EXPERIMENTS

2:6 MATERIALS

2:6.1 INCUBATION MEDIA

2:6.1.1. Mammalian Ringer

Mammalian Ringer solution was prepared fresh daily by the addition of the appropriate volume of distilled water to a preweighed mixture of salts. The final concentration (meq/1) of each ion species was as follows (Burgen & Mitchell, 1968):
Na^+, 159.9; K^+, 5.6; Ca^{++}, 4.4; Cl^-, 163.9; HCO_3^-, 5.9; Glucose, 5.0. The pH of this solution was 7.4.

2:6.1.2. Sodium-free Ringer

Sodium-free Ringer solution was prepared according to two methods described by Horn (1974). With the first method, each sodium salt was replaced with the appropriate choline salt. Thus the ionic composition of the medium remained unchanged, except that choline ions had been substituted for sodium ions. With the second method, all sodium salts were simply omitted. This inevitably meant that the concentration of Cl^- and HCO_3^-, as well as Na^+, was changed. The final concentration (meq/1) of each ion species was thus as follows: Na^+, 0; K^+, 5.6; Ca^{++}, 4.4; Cl^-, 5.6; HCO_3^-, 0; Glucose, 5.0. To obtain an isotonic incubation medium using this latter method, the salts were dissolved in 0.32 M sucrose solution instead of distilled water (Horn, 1974).

The pH of sodium-free incubation medium was 7.4.

2:6.2 SCINTILLATION FLUID

The scintillation fluid contained 0.267% PPO (2,5-diphenoxyloxazole), 0.0067% POPOP (1,2,bis-(5-phenyloxazole)-benzene) in toluene with 33% Triton X-100. All reagents used were scintillator grade (British Drug Houses Limited).

2:6.3 RADIOACTIVE COMPOUNDS

The radioactive drug solutions used in the experiments were:

1. Noradrenaline. (-)-noradrenaline-carbinol ^{14}C-bitartrate, specific activity 5.0 mC/mmole; Radiochemical Centre Limited.
2. Mescaline. Mescaline-8-^{14}C-hydrochloride, specific activity 5.2 mC/mmole; New England Nuclear Corporation Inc.

2:7 EXPERIMENTAL METHODS

Synaptosomes were isolated and incubated according to a modification of the method described by Maxwell et al, (1974).

2:7.1 PREPARATION OF SYNAPTOSOMES

The procedure for the preparation of synaptosomes is outlined in Figure 8.

For each experiment, one male albino Wistar rat, weighing between 250 and 350 g was sacrificed by decapitation. The brain was quickly removed and the cerebral cortex dissected from the rest of the tissue on ice. The cerebral cortex was immediately homogenised in 0.32 M sucrose (10 ml) using a teflon pestle and glass vessel. The blood and cellular debris were separated by centrifugation at 1000 g for 10 min at 4°C. The supernatent was removed and centrifuged at 17000 g for 5 min at 4°C. A pellet was formed which was considered to be rich in synaptosomes (see Gray & Whittaker, 1961). This pellet was resuspended in cold mammalian Ringer solution (70 ml).

2:7.2 INCUBATIONS

An example of an experimental protocol is shown in Figure 9.

Synaptosome-rich Ringer solution (4.9 ml) (see 2:8.1) was added to each incubation flask. A 0.9% solution of NaCl (0.05 ml) was added to control flasks while a solution of the test substance (0.05 ml) was added to the test flasks. The flasks were pre-incubated at 37°C for five minutes in a shaking water bath (Gallenkamp)

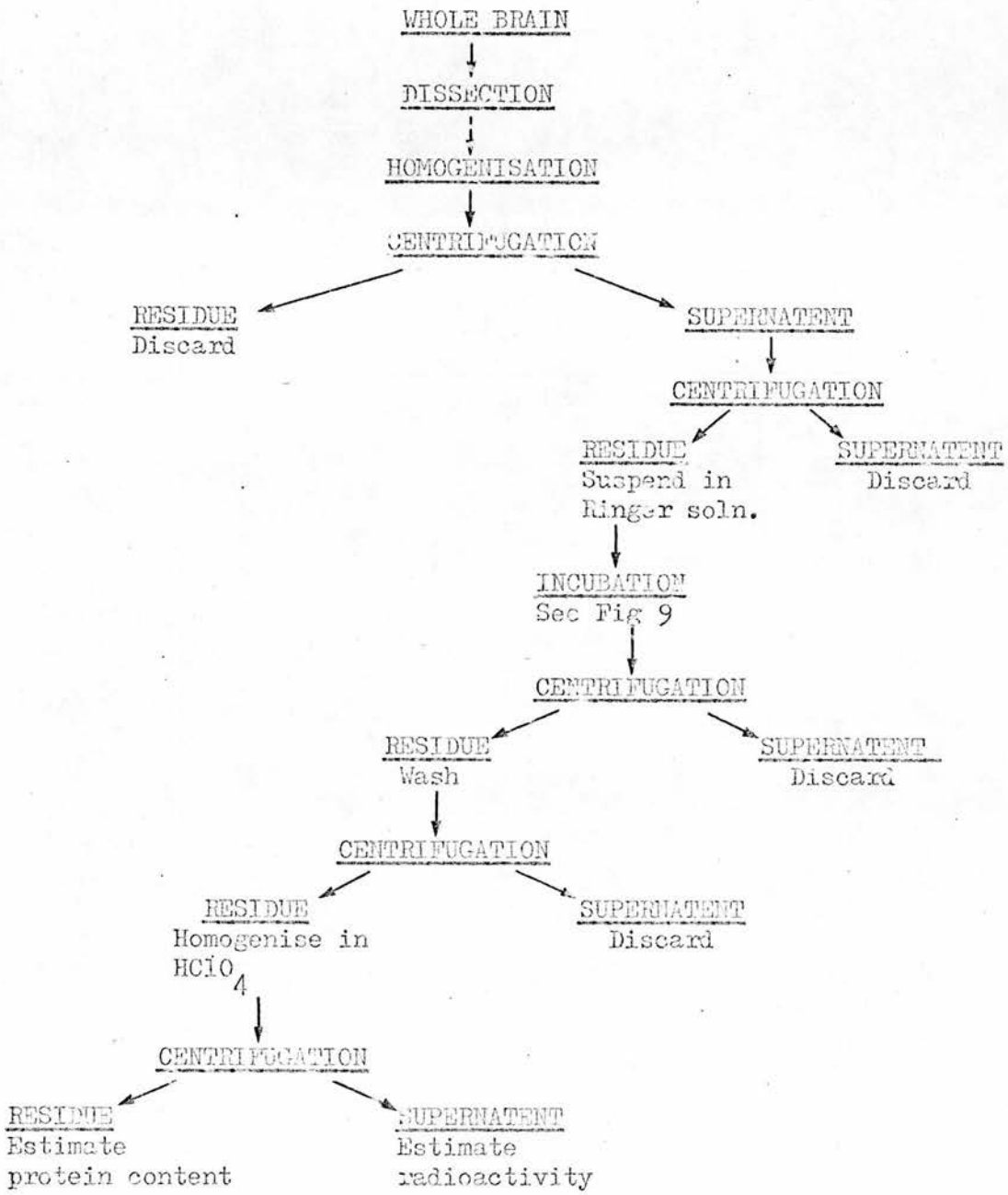


FIGURE 8 Schematic diagram of biochemical (uptake) experiments

after which a solution of ^{14}C-labelled monoamine (0.05 ml) was added to each flask. The incubations were then continued for a further 10 minutes.

The temperature and sodium dependent nature of the monoamine uptake were assessed by variations of the above experimental protocol. In order to determine the temperature-dependent nature of uptake, the pre-incubation and incubation temperatures were changed to 4°C (see, for example, Maxwell et al, 1974). The sodium dependency was investigated by performing the incubations in sodium-free Ringer solution (see 2:6.1.2).

Incubations were stopped by pouring the contents of each flask into chilled tubes, followed by the immediate centrifugation at 15000 g for 5 min at 4°C. The resulting (synaptosome-rich) pellet was washed in 0.9% NaCl (5 ml) and the centrifugation repeated. The supernatent from the wash was discarded and the pellet blotted dry.

2:7.3 ASSAY PROCEDURES

Standard biochemical techniques were employed for the estimation of radioactivity and protein concentration in each incubation sample.

2:7.3.1. Estimation of ^{14}C-monoamine content

The washed pellet (2:7.2) was homogenised in 0.4 N perchloric acid (1 ml) and the homogenate allowed to stand for 30 minutes. The protein thus precipitated was removed by centrifugation at 15000 g for 5 min at 4°C. An aliquot of the supernatant (0.5 ml) was added to a glass scintillation vial, containing 1N NaOH (0.2 ml) to neutralise the acid, and scintillation fluid added (10 ml). Radioactivity

ISOTOPE ^{14}C-mescaline SPECIFIC ACTIVITY 5.2 mCi/mmole

INHIBITOR Desipramine (DMI)

PRE-INCUBATION: 5 MINS

INCUBATION: 10 MINS @ 37 °C

NO.	ISOTOPE CONCN (μM)	CONTROL	TEST
1	1.000	0.9% NaCl	0.05 μM DMI
2	0.500	"	"
3	0.200	"	"
4	0.100	"	"
5	0.066	"	"
6	0.050	"	"

FIGURE 9 Example of a biochemical experimental protocol

6 incubation flasks were prepared in duplicate. "Control" flasks contained 0.9% NaCl (0.05 ml) whereas "Test" flasks contained 0.05 μM desipramine (0.05 ml). To each flask was added synaptosome-rich Ringer solution and the radioactive moncamine solution (see 2:7.2).

was measured in a Packard liquid scintillation spectrometer, where counting efficiency was monitored by the channel ratio method (Red channel: gain 8%, discriminator 50 - 125V; Green channel: gain 8%, discriminator 50 - 1000 V).

2:7.3.2. Estimation of Protein Content

The precipitated protein (2:7.3.1) was dissolved by heating for 30 min in 2N NaOH (5 ml) at 100°C. The protein content of this solution was estimated according to the method described by Lowry, Rosebrough, Farr and Randall (1951). Bovine serum albumin was used to construct standard curves for this estimation.

2:8 ESTIMATION OF THE PARAMETERS OF MONOAMINE UPTAKE KINETICS

2:8.1 DEFINITIONS

Monoamine "uptake" is defined as the effect of a process whereby a monoamine is accumulated into synaptosomes. The uptake velocity is the number of moles of monoamine accumulated by each incubation sample (2:7.3.1) corrected for the protein concentration (2:7.3.2) and for the time of incubation. Thus the uptake velocity is expressed as pmoles/min/mg protein.

The terms Vm and Km are defined on the basis of enzyme kinetics. Vm is the maximum uptake velocity of the system being studied, whereas Km is the monoamine concentration which gives rise to the half-maximal uptake velocity (Mahler & Cordes, 1966).

2:8.2 CALCULATION OF Vm AND Km

For both monoamines examined, the uptake velocity was determined for different concentrations of the monoamine in the incubation medium. The data were analysed using regression analysis. In the case of data which demonstrated Michaelis-Menten type kinetics, the analyses were performed using the method described by Wilkinson (1961). This method provides accurate estimates for the Vm and Km. In the case of experiments performed in the presence of an inhibitor, the method provides estimates for the apparent Vm and apparent Km.

Thus, by statistically comparing the Vm and Km with the apparent Vm and apparent Km (see below), the effect

of the inhibitor was established. The nature of the inhibition was defined on the basis of enzyme kinetics (see Results) (Mahler & Cordes, 1966).

2:8.3 STATISTICAL TESTS

For both monoamines examined, an estimate of Km and Vm was calculated according to the method of Wilkinson (1961). Using this method, the standard error of the estimate was also calculated for Km and Vm.

The statistical significance of the difference between two estimates was evaluated using Student's t-test. Levels of probability were estimated from the two-tailed Student's t-distribution.

Two estimates were considered as differing significantly if the probability of the difference occurring at random was less than 2%. (i.e. $p < 0.02$).

CHAPTER 3

RESULTS

3:1 RESPONSES OF SINGLE NEURONES TO PUTATIVE NEUROTRANSMITTERS

3:1.1 INTRODUCTION

The postulated transmitter role of the monoamines, and their implication in the action of psychotropic drugs, forms the background to the many investigations into the sensitivity of central neurones to NA, DA and 5HT. Both excitatory and inhibitory responses of single cortical neurones to NA and 5HT (Bradshaw et al, 1971a; 1973a; 1974; Bevan et al, 1974a) and to DA (Stone, 1974) have been reported. Similarly, both excitatory and depressant responses to NA and DA have been reported in the caudate nucleus (Spencer & Havlicek, 1974). However, there is considerable disagreement between different research groups concerning the true nature of the response to each monoamine. Depressant responses have been attributed to the use of barbiturate anaesthesia (Johnson et al, 1969b). It has been suggested that excitatory responses are artefacts produced by the expulsion of hydrogen ions from acidic drug solutions (Jordan et al, 1972a). Excitatory responses have also been attributed to an anoxia produced by the vasoconstrictor action of monoamines on blood vessels adjacent to the site of recording (Stone, 1972).

As a preface, then, to the description of the drug-interaction studies, a survey of the effects of putative neurotransmitters on single neurones in the cerebral cortex and caudate nucleus of the rat is reported.

TABLE 1

Responses of cortical neurones to monoamines and acetylcholine

Agonist	Response (number of cells)		
	Excitation	Depression	Biphasic
Dopamine	20 (50%)	17 (43%)	3 (7%)
Noradrenaline	23 (47%)	20 (41%)	6 (12%)
5-hydroxytryptamine	8 (57%)	5 (36%)	1 (7%)
Acetylcholine	22 (100%)	0 (0%)	0 (0%)

3:1.2 RESPONSES OF SINGLE NEURONES IN THE CEREBRAL CORTEX TO THE MONOAMINES AND ACETYLCHOLINE

The effects of DA, NA, 5HT and ACh were studied on 125 spontaneously active neurones in the cerebral cortex. Three types of responses could be observed: 1. excitation; 2. depression; and 3. biphasic responses, consisting of an initial depressant phase followed by an excitatory phase. Table 1 shows the frequency of occurrence of these effects.

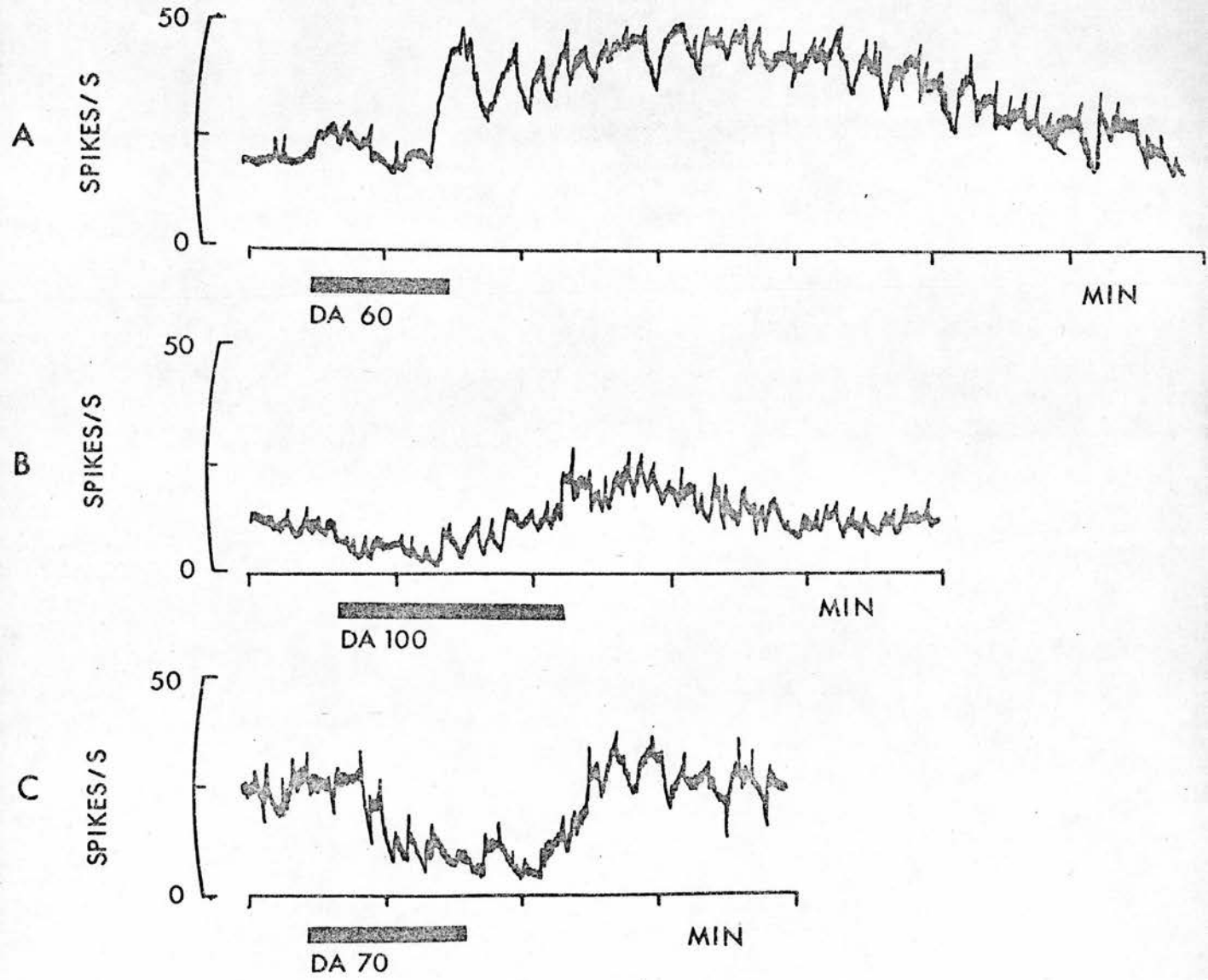
In order to determine whether an agonist had produced predominantly excitatory or depressant effects, the numbers of excitations and depressions observed in response to each agonist were examined using the Binomial test. (Biphasic responses were not considered in this analysis.) The Null hypothesis was that the probability of observing an excitatory response is 0.5. For both NA and DA, the Null hypothesis was upheld: there was an equal probability of observing either excitatory or depressant responses. In the case of 5HT and of ACh, there was a significantly greater probability of observing excitatory responses than of observing depressant responses ($p = 0.005$ and 0.002 for 5HT and ACh respectively).

3:1.3 RESPONSES OF SINGLE NEURONES IN THE CAUDATE NUCLEUS TO THE MONOAMINES AND ACETYLCHOLINE

The effects of DA, NA, 5HT and ACh were studied on 208 spontaneously active neurones in the caudate nucleus. As in the cerebral cortex, three types of responses to each agonist could be observed: 1. excitation; 2. depression; and 3. biphasic responses. Figure 10 shows an example of each type of response to the microelectrophoretic

FIG. 10. Examples of the types of response to dopamine (DA) observed in these experiments. Each excerpt is taken from rate meter recordings of the firing rate of a single neurone in the caudate nucleus. Ordinates: firing rate (spikes/s); abscissae: time (minutes). Horizontal bars indicate DA applications; numbers refer to intensity of ejecting current (nA).

(A) Excitatory response. (B) Biphasic response (depressant phase followed by excitatory phase). (C) Depressant response.


A
50
SPIKES/S
0
DA 60
MIN
B
50
SPIKES/S
0
DA 100
MIN
C
50
SPIKES/S
0
DA 70
MIN

application of DA; Table 2 shows the frequency of occurrence of each type of response for all four agonists.

In order to determine whether an agonist had produced predominantly excitatory or depressant effects, the numbers of excitations and depressions observed in response to each agonist were examined using the Binomial test (see 3:1.2). Biphasic responses were not considered in this analysis. The Null hypothesis was that the probability of observing an excitatory response is 0.5. In the case of DA and NA, there were no significant differences ($p > 0.05$) between the numbers of excitations and depressions actually observed and the numbers predicted by the Null hypothesis. However, there was a significant increase in the number of excitatory responses to 5HT ($p = 0.005$) and ACh ($p = 0.002$) compared with the numbers predicted by the Null hypothesis.

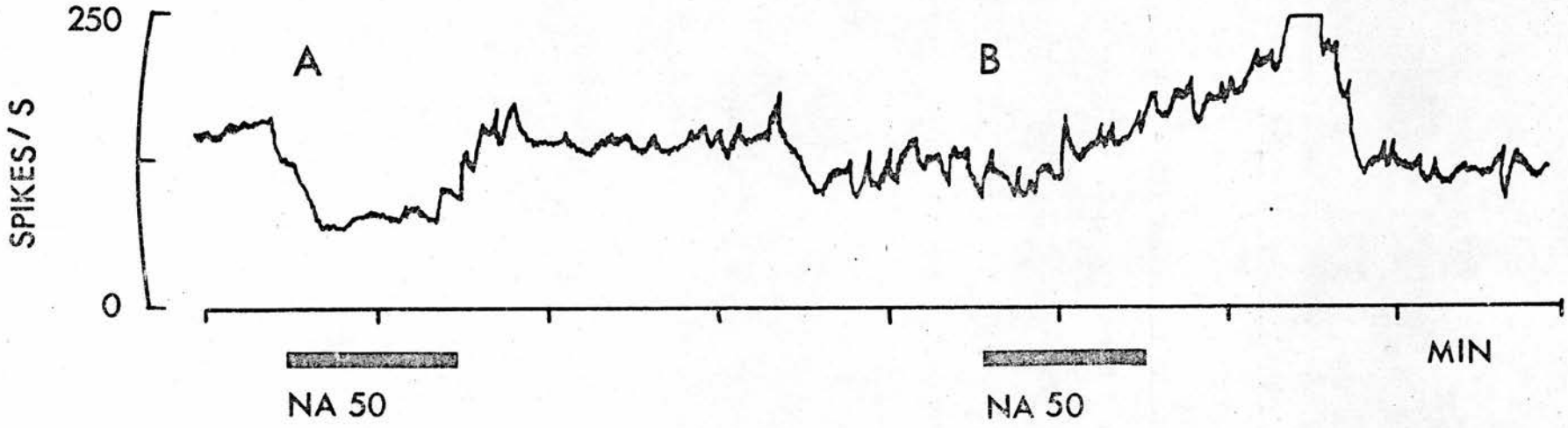
On eight cells a "spontaneous reversal" of the response to the monoamine could be observed: the cell first responded with a clear depression or excitation to the monoamine; later, however, the same monoamine evoked an opposite response. "Spontaneous response reversal" was observed on four cells with DA, two cells with NA and on two cells with 5HT. An example of spontaneous reversal is shown in Figure 11. This cell was initially depressed by the application of NA (50nA for 60 sec). However, the subsequent application of NA evoked an excitatory response. Cells showing "spontaneous response reversal" were not used for drug interaction studies.

TABLE 2

Responses of caudate neurones to monoamines and acetylcholine

Agonist	Response (number of cells)		
	Excitation	Depression	Biphasic
Dopamine	33 (53%)	21 (34%)	8 (13%)
Noradrenaline	32 (50%)	26 (41%)	6 (9%)
5-hydroxytryptamine	32 (64%)	12 (24%)	6 (12%)
Acetylcholine	52 (91%)	4 (7%)	1 (2%)

FIG. 11. Spontaneous response reversal. Ratemeter recording of the firing rate of a single neurone in the caudate nucleus. Ordinate: firing rate (spikes/s); abscissa: time (minutes). Horizontal bars indicate noradrenaline (NA) applications; numbers refer to the intensity of the ejecting current (nA). (A) Depressant responses to NA. (B) Subsequential application of NA evoked an excitatory response.


250
0
SPIKES / S
A
B
NA 50
NA 50
MIN

3:1.3.1. Correlation between responses to dopamine and responses to other agonists

On 48 cells in the caudate nucleus responses to DA and to another agonist (NA, 5HT, ACh) were compared. (On a number of cells, responses to DA and to two other agonists were tested.) Table 3 shows the correlation between the direction of the response (excitation or depression) to DA and the direction of the response to the other agonist. It is apparent that there was a highly significant correlation between the effects of DA and NA, whereas there was no significant correlation between the effects of DA and 5HT. Furthermore, there was no significant correlation between responses to DA and ACh (χ^2 test).

3:1.4 DISCUSSION

Other authors have described the occurrence of excitatory and depressant responses to DA (Gonzalez-Vegas, 1974; Siggins et al, 1974; Spencer & Havlicek, 1974) and to NA (Spencer & Havlicek, 1974) in the caudate nucleus of the rat. Similarly, both excitatory and depressant responses to NA, DA and 5HT (Stone, 1973a; 1973b) and to DA (Stone, 1974) have been described in the cerebral cortex of the rat. Furthermore, both excitatory and depressant responses to NA, DA and 5HT have been described in the cerebral cortex and caudate nucleus of the cat (see Curtis & Crawford, 1968).

In the experiments described above both excitatory and depressant responses to NA, DA and 5HT were observed in the cerebral cortex and caudate nucleus of the rat. With the exception of 5HT, there was no significant difference

TABLE 3

Correlation between the effects of dopamine and of other agonists on caudate neurones

Agonists compared *		Direction of responses to the two agonists		Significance of correlation (X^2 test)
		Same	Opposite	
Dopamine, Noradrenaline	(33)	91%	9%	$p < 0.001$
Dopamine, 5-hydroxytryptamine	(19)	79%	21%	N.S.
Dopamine, Acetylcholine	(17)	71%	29%	N.S.

* Figures in parenthesis indicate number of cells on which comparisons were made.

between the number of excitatory and of depressant responses to a monoamine; there was an equal probability of observing excitatory and depressant responses to both NA and DA. In the case of 5HT, however, excitatory responses predominated in both the cerebral cortex and caudate nucleus.

Some neurones responded in a biphasic fashion to the monoamines (see Tables 1 and 2). Biphasic responses to the monoamines have been reported in the cerebral cortex of the cat (Johnson et al, 1969a; Szabadi & Bradshaw, 1974) and in the caudate nucleus of both the rat (York, 1970) and the monkey (York, 1972). The presence of biphasic responses, and the observation of spontaneous response reversal, may suggest that both excitatory and inhibitory receptors to the same monoamine can co-exist on the same cell (Szabadi & Bradshaw, 1974).

In the caudate nucleus, there was a high correlation between both excitatory and depressant effects of DA and NA, whereas there was no significant correlation between the effects of DA and 5HT (see Table 3). This suggests that DA and NA may act at similar receptors on caudate neurones. Indeed, it is known that DA can stimulate α-receptors in the periphery (Rossum, 1965).

Both excitatory and inhibitory responses to ACh have been reported in the cerebral cortex (Stone, 1973) and caudate nucleus (Spencer & Havlicek, 1974) of the rat, and similar results have been reported in the cerebral cortex (Crawford & Curtis, 1966) and caudate nucleus (McLennan & York, 1966) of the cat. However, in the experiments described above, ACh evoked exclusively excitatory responses

in the cerebral cortex, and almost invariably evoked excitatory responses when applied to single neurones in the caudate nucleus.

When, in the caudate nucleus, responses to DA and ACh were compared on the same neurone, no significant correlation could be found between the directions of the responses to the two agonists (see Table 3). This would argue against the claim that DA and ACh have opposite effects in the caudate nucleus (Klawans, 1968; Yahr & Duvoisin, 1972).

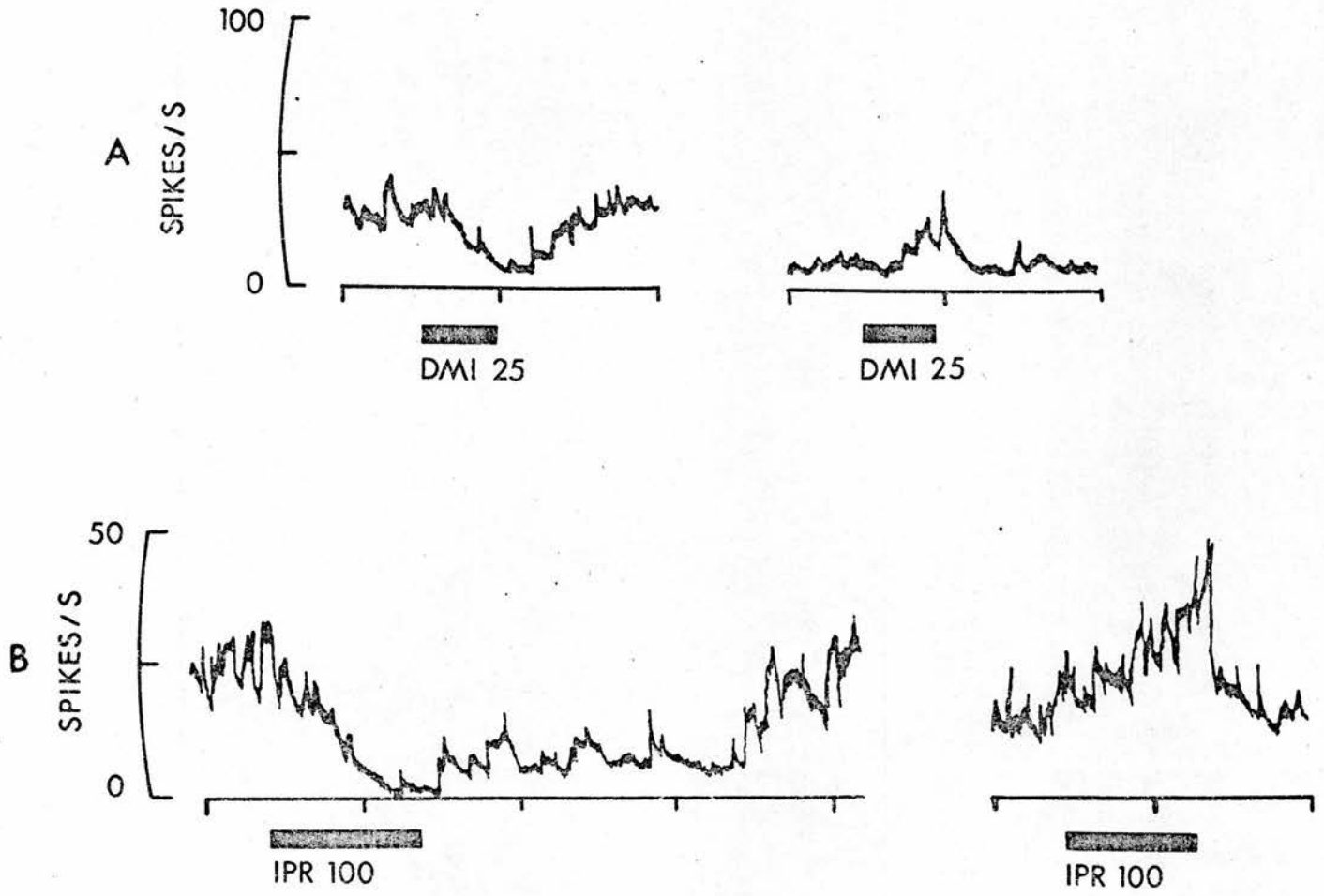
3:2 THE EFFECTS OF DESIPRAMINE ON RESPONSES OF SINGLE CAUDATE NEURONES TO MONOAMINES AND ACETYLCHOLINE

3:2.1 INTRODUCTION

It is generally believed that the symptomatology of Parkinson's disease is caused by the selective degeneration of the dopamine-containing nigrostriatal pathway which leads to an impaired balance between dopaminergic and cholinergic inputs in the striate nucleus (Barbeau, 1962; Klawans, 1968). Drugs effective in the treatment of Parkinson's disease are thought to act either by inhibiting the effects of acetylcholine (eg. atropine), or by enhancing the effects of dopamine (eg. L-DOPA) (Klawans, 1968; Yahr & Duvoisin, 1972).

It has been reported that desipramine, a tricyclic antidepressant drug, is effective in the treatment of Parkinsonism (Laitenen, 1969). The basis for the anti-Parkinsonian efficacy of desipramine, however, is not known. Although the anticholinergic effects of desipramine are well documented (Atkinson & Ladinsky, 1972) it is not likely that this is the sole explanation for the anti-Parkinsonian efficacy of the drug, since the therapeutic effectiveness of desipramine is greatly enhanced by the concurrent administration of another anticholinergic (Yahr & Duvoisin, 1972). Another possibility could be that desipramine potentiates the effects of dopamine (DA) in the caudate nucleus. Such an effect, however, could not be predicted on the basis of the well known 'uptake blockade hypothesis of potentiation' (Iversen, 1974), since desipramine is almost completely ineffective in blocking the

FIG. 12. Effect of desipramine and iprindole on the firing rate of single neurones. The figure shows excerpts from the ratemeter recording of the firing rate of 4 single neurones. Ordinates: firing rate (spikes/s); abscissae: time (minutes). Each excerpt is from a different neurone. Horizontal bars indicate applications of a tricyclic antidepressant drug. (A) Depressant (left) and excitatory (right) effect of desipramine (DMI) on the firing rates of single caudate neurones. (B) Depressant (left) and excitatory (right) effect of iprindole (IPR) on the firing rates of single cortical neurones.


A
100
SPIKES/S
0
DMI 25
DMI 25
B
50
SPIKES/S
0
IPR 100
IPR 100

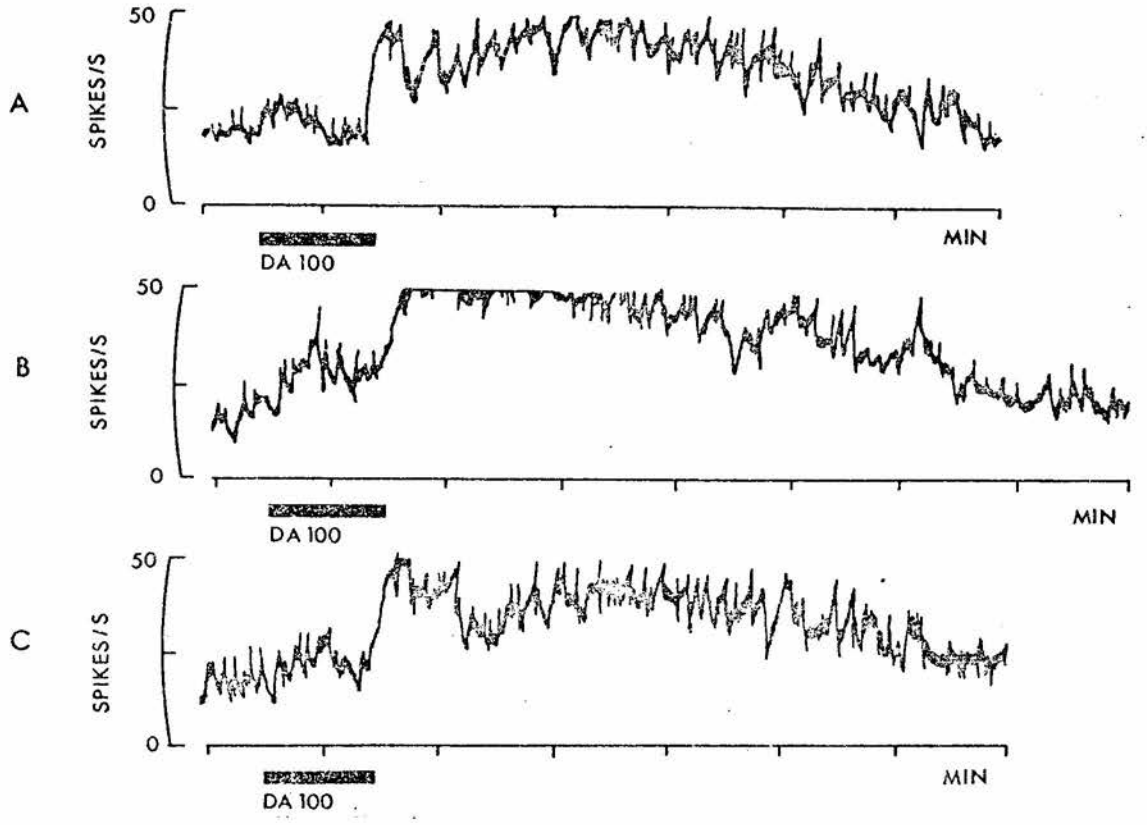
uptake of NA in the caudate nucleus (Ross & Renyi, 1967; Horn et al, 1971).

It has been reported that desipramine has a dual effect on neuronal responses to noradrenaline (NA), 5-hydroxytryptamine (5HT) and acetylcholine (ACh) in the cerebral cortex: both antagonism and potentiation of the responses can be observed (Bradshaw et al, 1971a; 1974; Bevan et al, 1973c; 1975a). In the experiments reported here, the technique of microelectrophoresis was used in order to examine how, in the caudate nucleus of the rat, neuronal responses to DA, NA, 5HT and ACh could be affected by desipramine.

3:2.2 EFFECT OF DESIPRAMINE ON NEURONAL FIRING

The direct effect of desipramine on the firing rate was studied on 65 cells. The dose of antidepressant applied was 30 - 100 nA passed for 30 - 80 seconds. On 6 cells (9%) the firing rate was increased during the application of desipramine, whereas on 19 cells (20%) the firing rate was decreased. There was no significant correlation between the dose of desipramine applied and the effect on neuronal firing. An example of both the excitatory and depressant effects of desipramine on the firing rate is shown in Figure 12A. On occasions a reduction in spike amplitude was observed; such cells were not used for drug interaction studies.

FIG. 13. Potentiation of excitatory responses of a single caudate neurone to dopamine (DA) by desipramine (DMI). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone. Horizontal bars indicate applications of DA; numbers refer to the intensity of the ejecting current (nA). (A) Control response to DA. (B) Potentiated response to DA one minute after a brief application of DMI (40 nA; 80 seconds). Recovery of control response 25 minutes after the application of DMI. The graph at the bottom shows the time-course of the entire study. The sizes of the responses to DA are expressed as a percentage of the mean of the control responses. Each column represents a single response. Letters above the graph indicate responses illustrated in the ratemeter tracings above.


A
B
C
SPIKES/S
50
0
MIN
DA 100

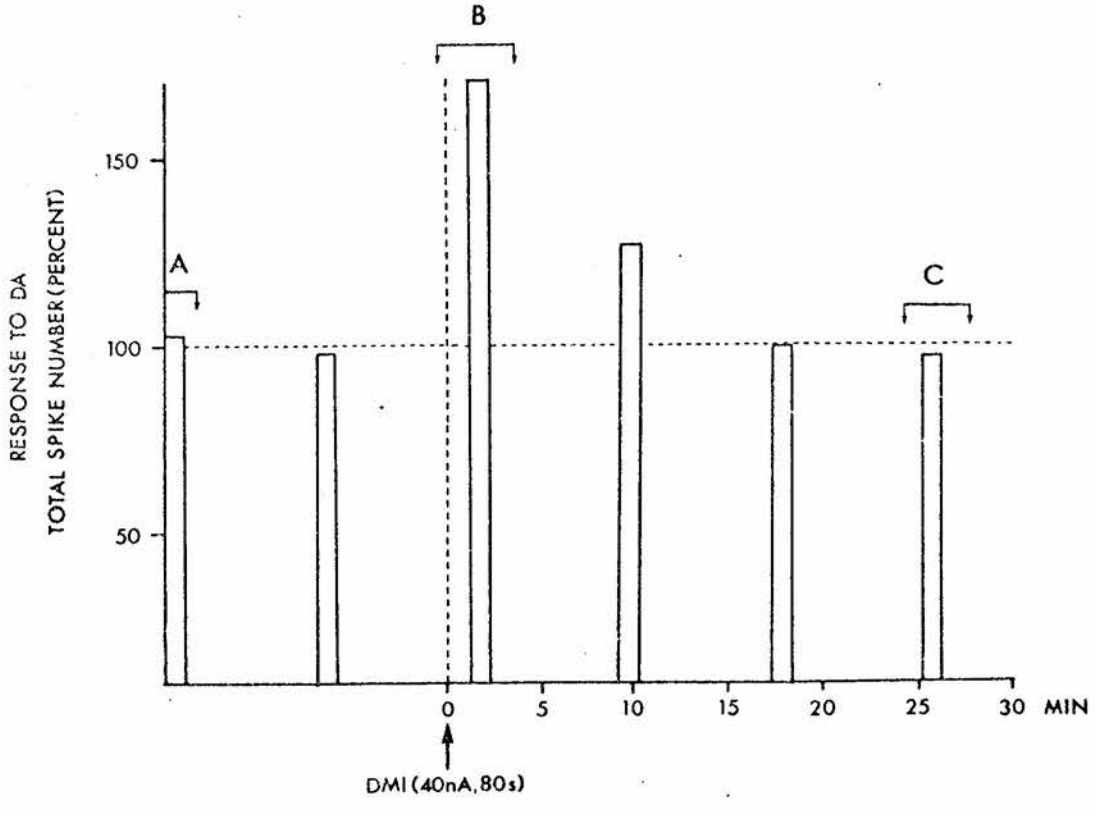
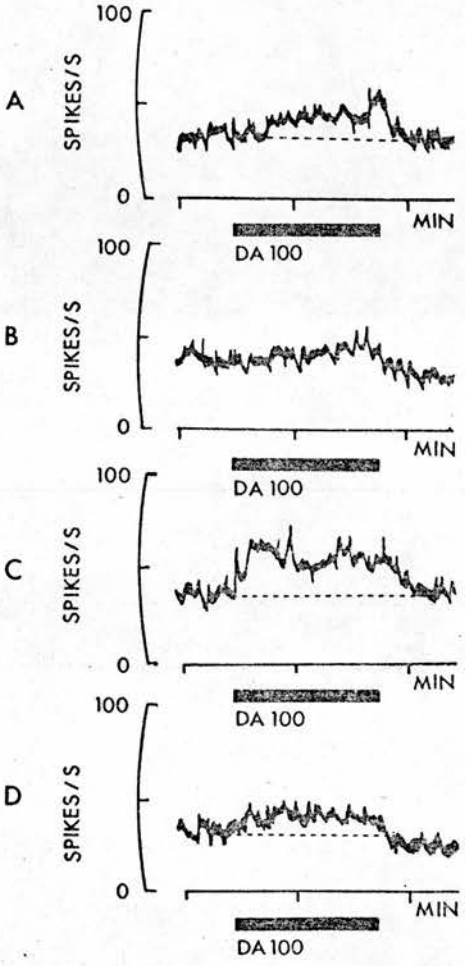
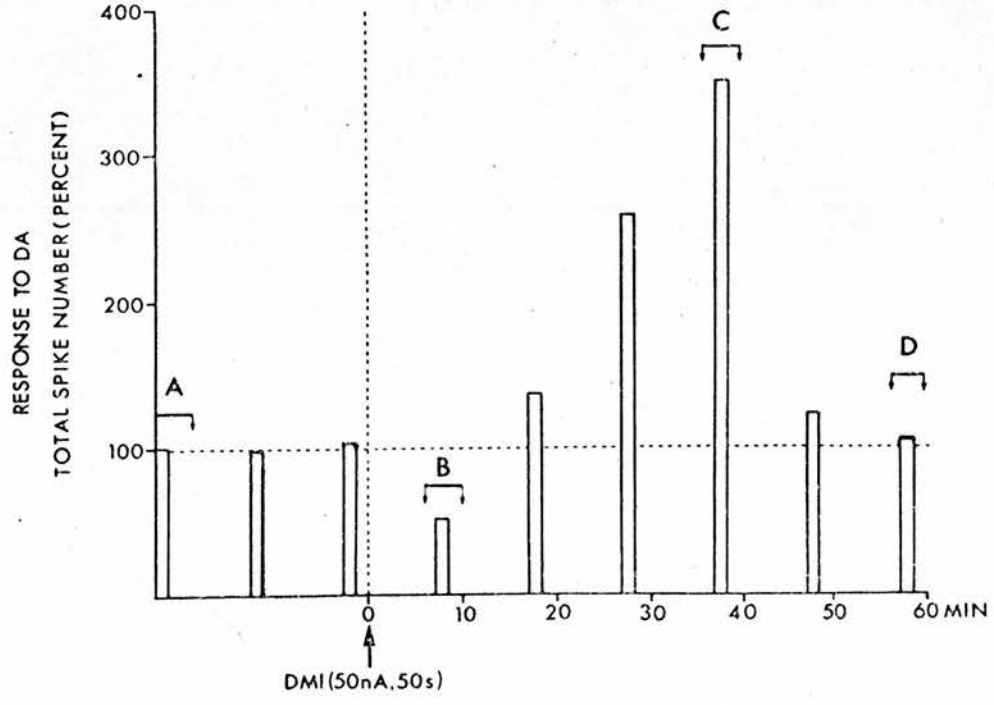

B
A
C
150
100
50
RESPONSE TO DA
TOTAL SPIKE NUMBER (PERCENT)
0
5
10
15
20
25
30
MIN
DMI (40nA, 80s)

FIG. 14. Antagonism and potentiation of excitatory responses of a single caudate neurone to dopamine (DA) by desipramine (DMI). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to DA. (B) Antagonised response, 8 minutes after a brief application of DMI (50 nA; 50 seconds). (C) Potentiated response, 38 minutes after the application of DMI. (D) Recovery of the control response 58 minutes after the application of DMI. Graph at the bottom shows the time-course of the entire study (as in Fig. 13).


A
B
C
D
100
0
SPIKES/S
MIN
DA 100


RESPONSE TO DA
TOTAL SPIKE NUMBER (PERCENT)
400
300
200
100
A
B
C
D
0
10
20
30
40
50
60 MIN
DMI (50nA, 50s)

3:2.3 EFFECT OF DESIPRAMINE ON RESPONSES TO DOPAMINE

3:2.3.1. Excitatory responses

Both potentiation and antagonism of excitatory responses to DA could be observed after a brief application (30 - 100 nA for 30 - 80 seconds) of desipramine.

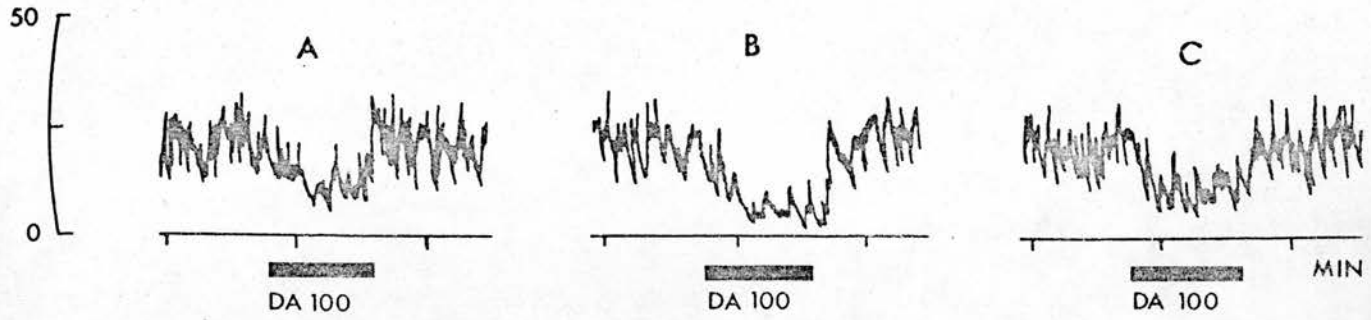
Potentiation of the response was seen on 5 cells. An example of potentiation is shown in Figure 13. Antagonism of the response to DA was seen on 6 cells. On 3 cells, both potentiation and antagonism could be observed. On each of these cells the response was first antagonised following the application of desipramine; this antagonism was followed later by potentiation and finally by recovery of the control response. An example of this dual effect of desipramine on excitatory responses to DA is shown in Figure 14. On 2 cells, no significant change in the size of the response could be observed after the application of desipramine. The degrees of potentiation and antagonism observed on each cell are summarised in Figure 20.

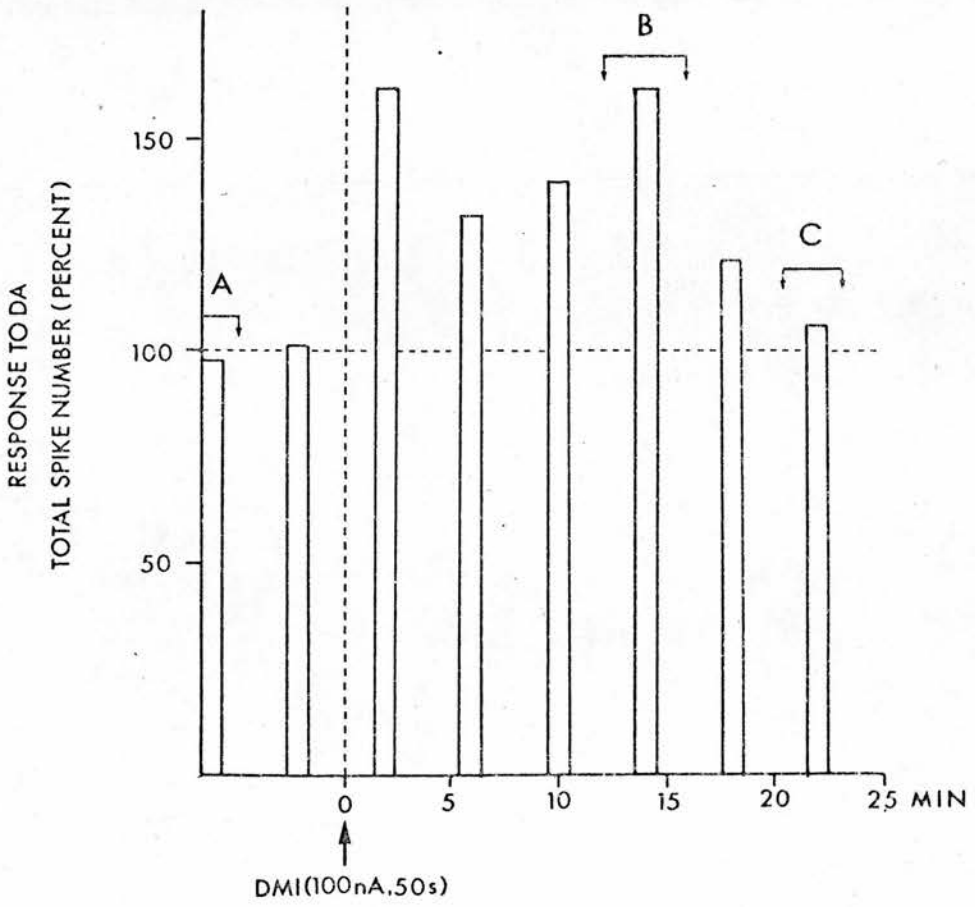
On another 3 cells, the excitatory response to DA was reversed into a depressant response following the application of desipramine.

3:2.3.2. Depressant responses

Both potentiation and antagonism of depressant responses to DA could be observed after a brief application of desipramine. Potentiation was seen on 6 cells, antagonism was seen on 3 cells. On one cell both antagonism and potentiation could be observed. An example of the potentiation of the depressant response to DA by desipramine

FIG. 15. Potentiation of depressant responses of a single caudate neurone to dopamine (DA) by desipramine (DMI). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to DA. (B) Potentiated response to DA 13 minutes after a brief application of DMI (100 nA; 50 seconds). (C) Recovery of the control response 21 minutes after the application of DMI. Graph at the bottom shows the time-course of the entire study (as in Fig. 13).


50
0
A
B
C
DA 100
DA 100
DA 100
MIN


RESPONSE TO DA
TOTAL SPIKE NUMBER (PERCENT)
150
100
50
A
B
C
0
5
10
15
20
25 MIN
DMI(100nA,50s)

is shown in Figure 15. On one cell no significant change in the size of the response could be observed after the application of desipramine. The degrees of potentiation or antagonism seen on each cell are summarised in Figure 20.

On 6 other cells, the depressant response to dopamine was reversed into an excitation following the application of desipramine.

3:2.4 EFFECT OF DESIPRAMINE ON RESPONSES TO NORADRENALINE

3:2.4.1. Excitatory responses

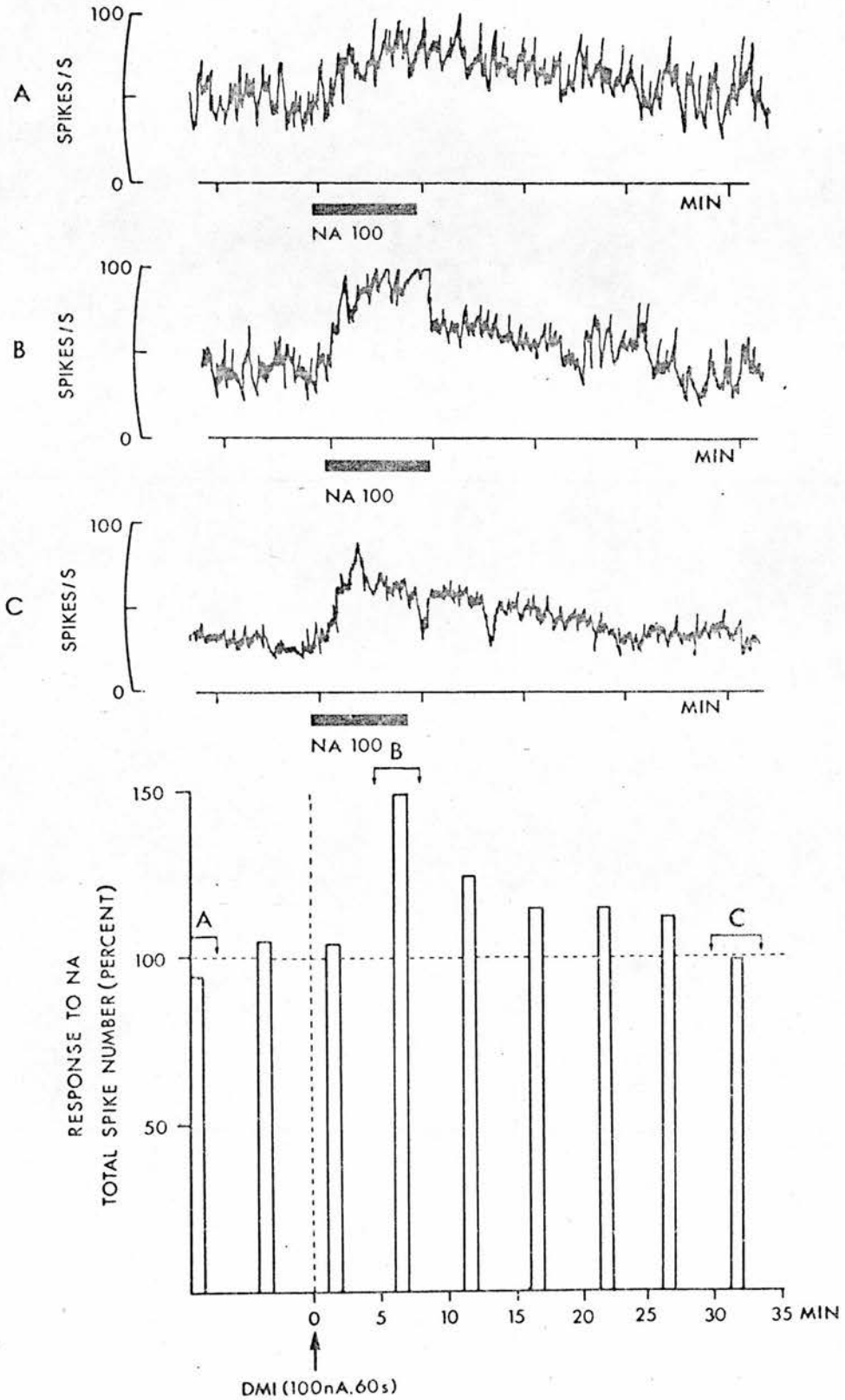
Both potentiation and antagonism of excitatory responses to NA could be observed after a brief application of desipramine. Potentiation was observed on 4 cells, antagonism was seen on 6 cells. On one cell both antagonism and potentiation could be observed, the antagonism preceding the potentiation. An example of the potentiating effect of desipramine on responses to NA is shown in Figure 16. On one cell no significant change in the size of responses could be observed after the application of desipramine. The degrees of potentiation or antagonism seen in each cell are summarised in Figure 20.

On one further cell the excitatory response to NA was reversed into a depression following the application of desipramine.

3:2.4.2. Depressant responses

Potentiation was seen on 6 cells depressed by NA, antagonism was observed on 4 cells. Both effects could be seen on 2 cells. On 2 cells no effect of desipramine could be observed. Figure 20 summarises the degrees of

FIG. 16. Potentiation of excitatory responses of a single caudate neurone to noradrenaline (NA) by desipramine (DMI). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig.13). (A) Control response to NA. (B) Potentiated response to NA 6 minutes after a brief application of DMI (100 nA; 60 seconds). (C) Recovery of the control response 31 minutes after the application of DMI. Graph at the bottom shows the time-course of the entire study (as in Fig. 13).


A
100
SPIKES/S
0
MIN
NA 100
B
100
SPIKES/S
0
MIN
NA 100
C
100
SPIKES/S
0
MIN
NA 100
B
150
A
100
C
50
RESPONSE TO NA
TOTAL SPIKE NUMBER (PERCENT)
0
5
10
15
20
25
30
35 MIN
DMI (100nA, 60s)

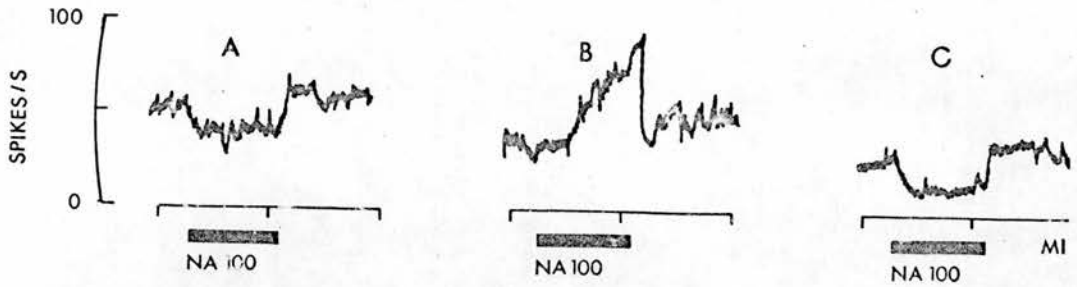


FIG. 17. Drug induced response reversal. Excerpts from the ratemeter recording of the firing rate of a single caudate neurone. Ordinate: firing rate (spikes/s); abscissae: time (minutes). Horizontal bars indicate the application of noradrenaline (NA); numbers refer to the intensity of the ejecting current (nA).

(A) Control response (depression) to NA.

(B) The response to NA was reversed into an excitation 45 minutes after the application of desipramine (50 nA; 50 seconds).

(C) Recovery of the depressant response to NA 160 minutes after the application of desipramine.

potentiation or antagonism observed on each cell.

On another 4 cells the depressant response to noradrenaline was reversed into an excitatory one following the application of desipramine. An example of a depressant response which was reversed following the application of desipramine is shown in Figure 17.

3:2.5 EFFECT OF DESIPRAMINE ON RESPONSES TO 5-HYDROXYTRYPTAMINE

3:2.5.1. Excitatory responses

Both potentiation and antagonism of excitatory responses to 5HT could be observed after a brief application of desipramine.

Potentiation of the response was observed on 4 cells. Antagonism of the response was seen on 3 cells. On 2 cells both antagonism and potentiation of the response could be seen; the antagonism preceded the potentiation. An example of the dual effect of desipramine on excitatory responses to 5HT is shown in Figure 18. On 2 cells no effect of desipramine could be observed. The data are summarised in Figure 20.

3:2.5.2. Depressant responses

On one cell, the depressant response to 5HT was reversed into an excitation, following the application of desipramine.

3:2.6 EFFECT OF DESIPRAMINE ON RESPONSES TO ACETYLCHOLINE

Both potentiation and antagonism of excitatory responses to ACh could be observed after a brief application

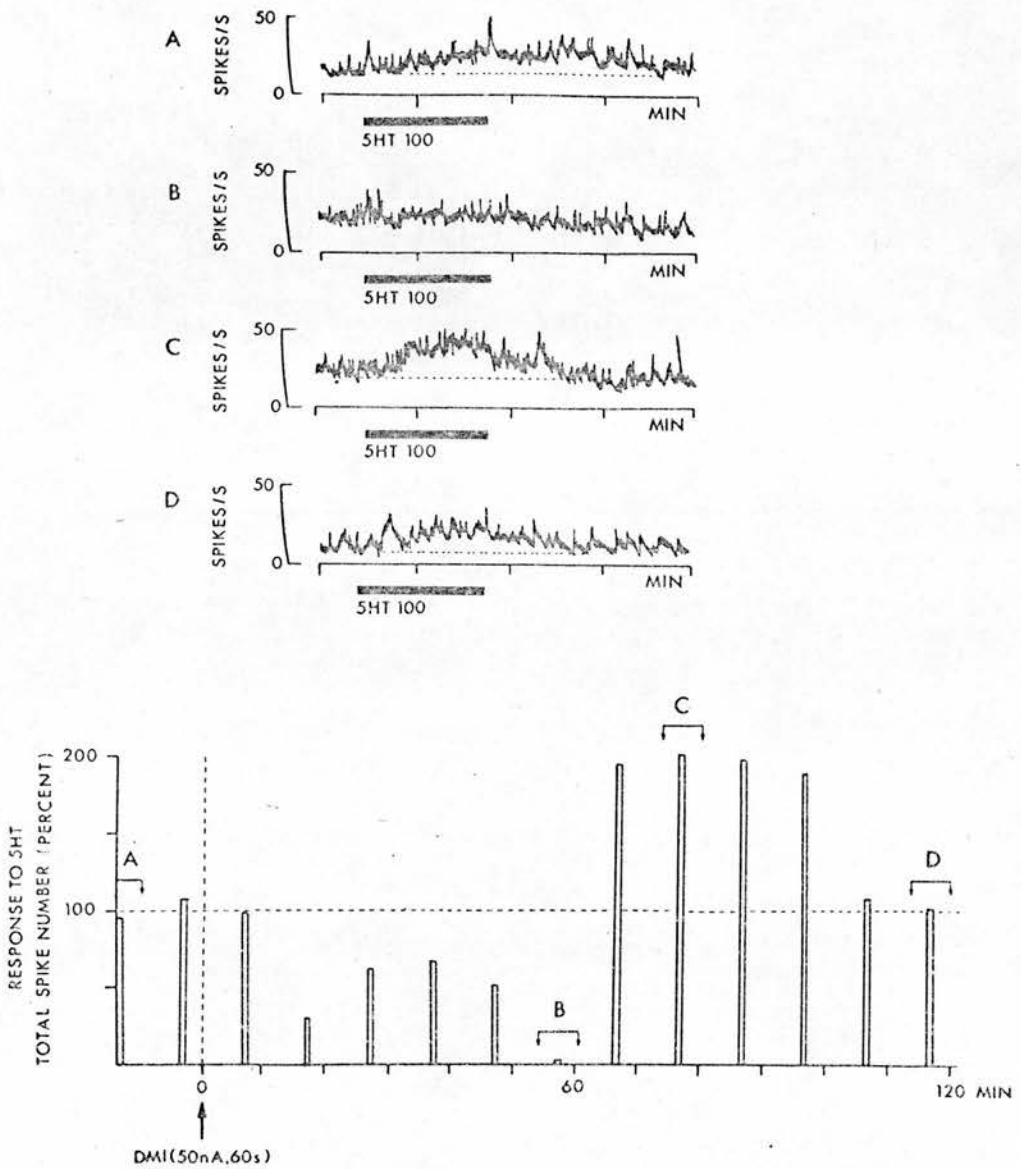


FIG. 18. Antagonism and potentiation of excitatory responses of a single caudate neurone to 5-hydroxytryptamine (5HT) by desipramine (DMI). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control responses to 5HT. (B) Antagonised response, 58 minutes after a brief application of DMI (50 nA; 60 seconds). (C) Potentiated response, 76 minutes after the application of DMI. (D) Recovery of the control response 116 minutes after the application of iprindole. Graph at the bottom shows the time-course of the entire study(as in Fig. 13).

of desipramine.

Potentiation was observed on 4 cells, antagonism was seen on 4 cells. On one cell both antagonism and potentiation could be seen; the antagonism preceded the potentiation. An example of the potentiating effect of desipramine on responses to ACh is shown in Figure 19. On 2 cells desipramine had no significant effect. The data are summarised in Figure 20.

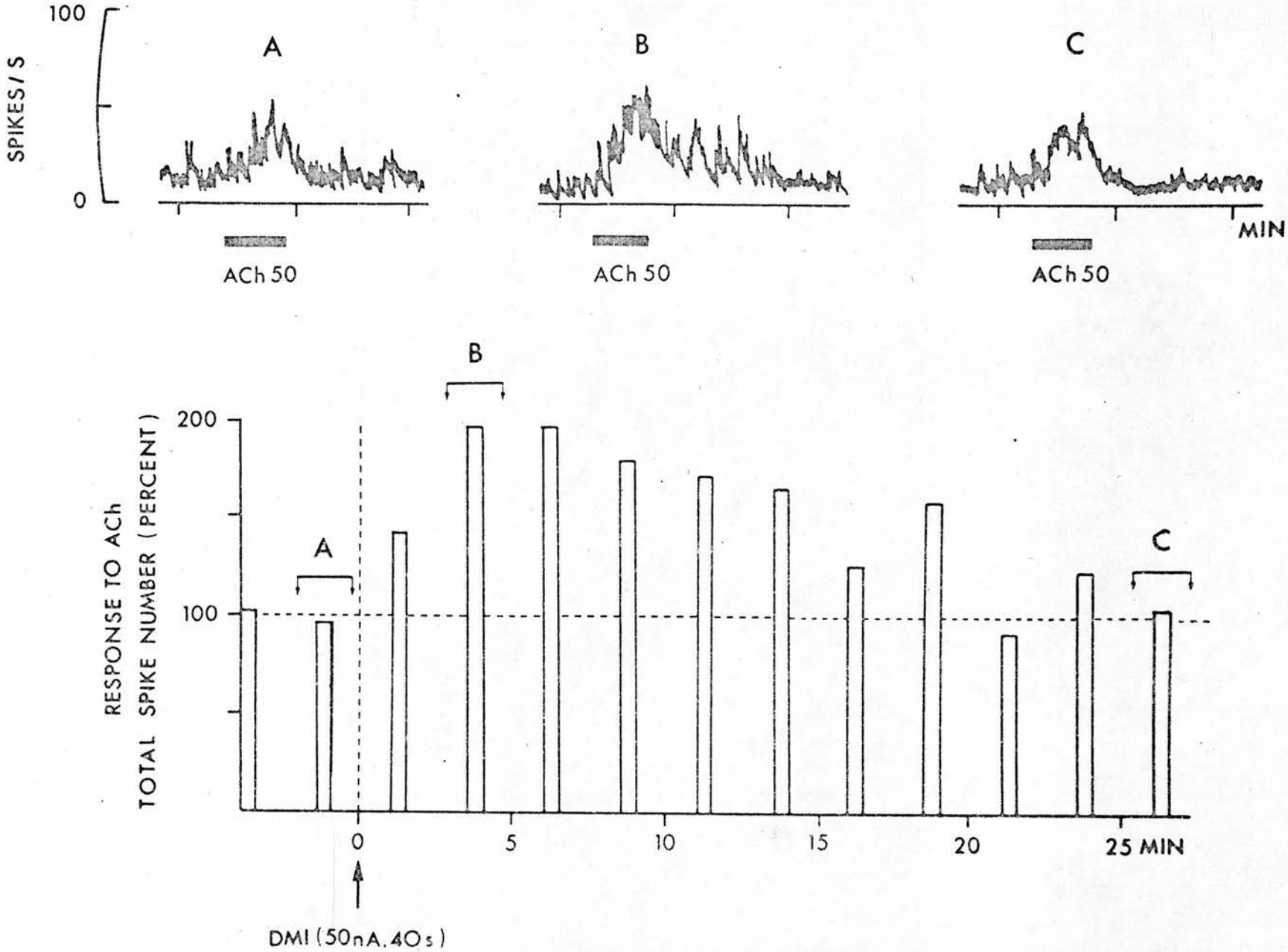
3:2.7 EFFECT OF DESIPRAMINE ON RESPONSES TO GLUTAMATE

The effect of desipramine on excitatory responses to glutamate was studied on 10 cells. The effects of glutamate on the firing rate were studied for 20 - 30 minutes following the application of desipramine. On none of the cells could any significant change be observed in the size of the responses to glutamate after the application of desipramine (see Figure 20). An example of a study of the effect of desipramine on responses of a single caudate neurone to glutamate is shown in Figure 21.

3:2.8 DISCUSSION

Desipramine applied by microelectrophoresis had a dual effect on responses to monoamines and ACh: both antagonism and potentiation of the responses could be observed. Responses to glutamate were not affected. A dual effect of desipramine on responses to NA and 5HT (Bradshaw et al, 1971a; 1974) and on responses to ACh (Bevan et al, 1973c; 1975a) has been described in the cerebral cortex of the cat.

FIG. 19. Potentiation of excitatory responses of a single caudate neurone to acetylcholine (ACh) by desipramine (DMI). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to ACh. (B) Potentiated response to ACh 4 minutes after a brief application of DMI (50 nA; 40 seconds). (C) Recovery of the control response 26 minutes after the application of DMI. Graph at the bottom shows the tine-course of the entire study (as in Fig. 13).


100
SPIKES / S
0
A
B
C
MIN
ACh 50
ACh 50
ACh 50
B
200
RESPONSE TO ACh
TOTAL SPIKE NUMBER (PERCENT)
100
A
C
0
5
10
15
20
25 MIN
DMI (50nA, 40s)

The antagonism of responses to NA could be interpreted on the basis of the α-adrenoceptor blocking effect of desipramine (Turker & Khairallah, 1967). Since receptors to NA and DA are likely to be very similar on caudate neurones, an α-receptor blocking action could explain the antagonism of responses to DA. The antagonism of responses to 5HT may be explained on the basis of the anti-serotonin action of the antidepressants (Domenjoz & Theobald, 1959), whereas the antagonism of responses to ACh may reflect the antimuscarinic action of these drugs (Atkinson & Ladinsky, 1972).

It is more difficult to interpret the potentiating effects of desipramine. In the case of 5HT, the most plausible explanation is uptake blockade. There is indirect evidence that 5HT terminals reach the caudate nucleus (Gumulka, Ramirez del Angel, Samanin & Valzelli, 1970), and that desipramine inhibits the uptake of 5HT into brain tissue (Ross & Renyi, 1969). Uptake blockade, however, cannot explain the potentiation of responses to DA and NA by desipramine. It is known that both DA and NA are accumulated by an active uptake process in the striatum (Horn et al, 1971); this uptake, however, is almost entirely unaffected by desipramine (Ross & Renyi, 1967; Horn et al, 1971).

The results presented here show that desipramine can potentiate neuronal responses to DA in the caudate nucleus. It is possible that this action is responsible for the therapeutic efficacy of desipramine in Parkinson's disease. However, responses to ACh can also be potentiated by

FIG. 20. Summary of the effects of desipramine on neuronal responses to dopamine, noradrenaline, 5-hydroxytryptamine (5HT), acetylcholine (ACh) and glutamate (GLU).

A. Potentiation: each point shows the maximum potentiation observed on one individaul cell (e.g. response B in Fig. 13). +: excitatory responses; —: depressant responses. A response was regarded as potentiated if there was more than 20% increase over the size of the control response (see 2:5.4).

B. Antagonism: each point shows the maximum degree of antagonism observed on one individual cell (e.g. response B in Fig. 14). A response was regarded as antagonised if there was more than 20% decrease over the size of the control response (see 2:5.4)

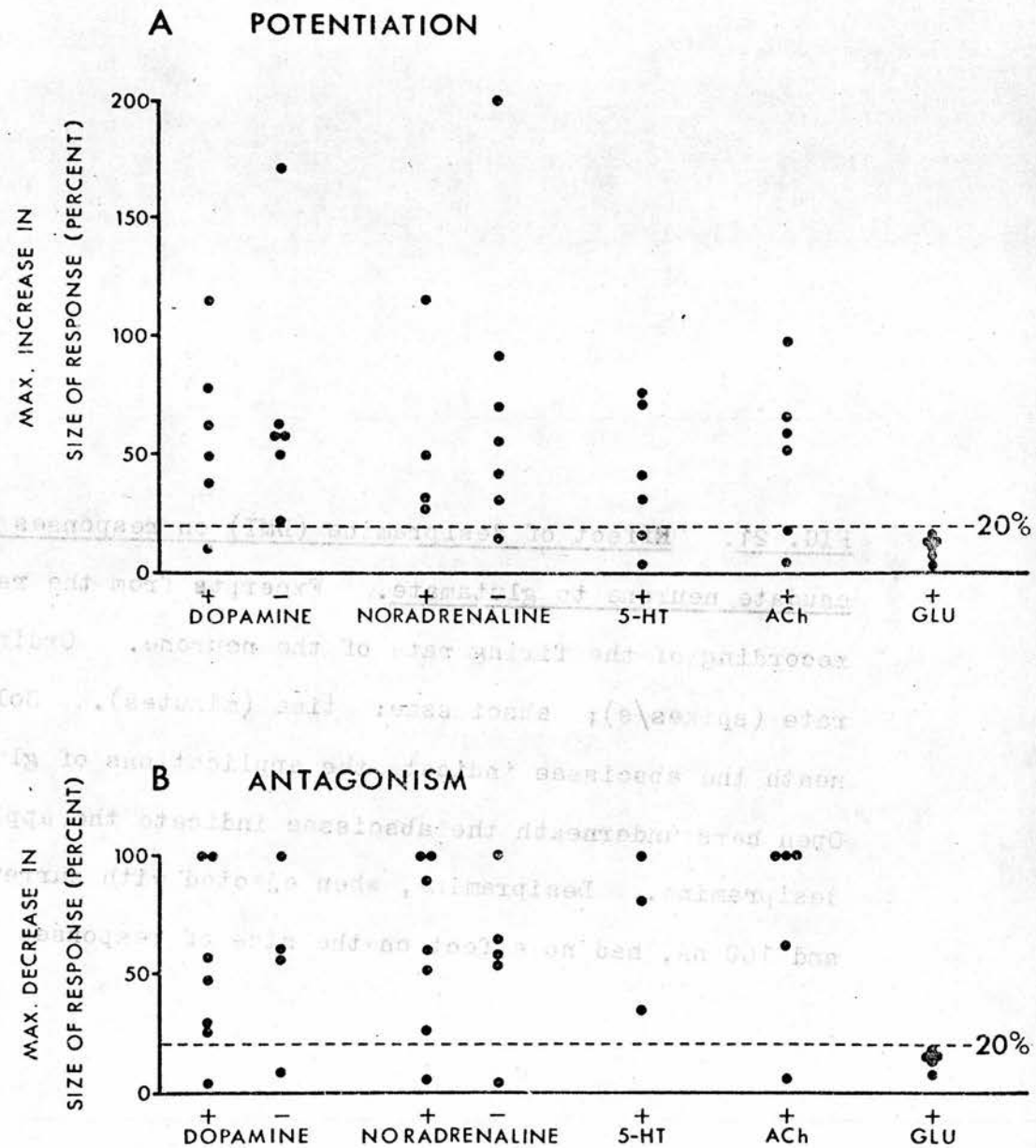
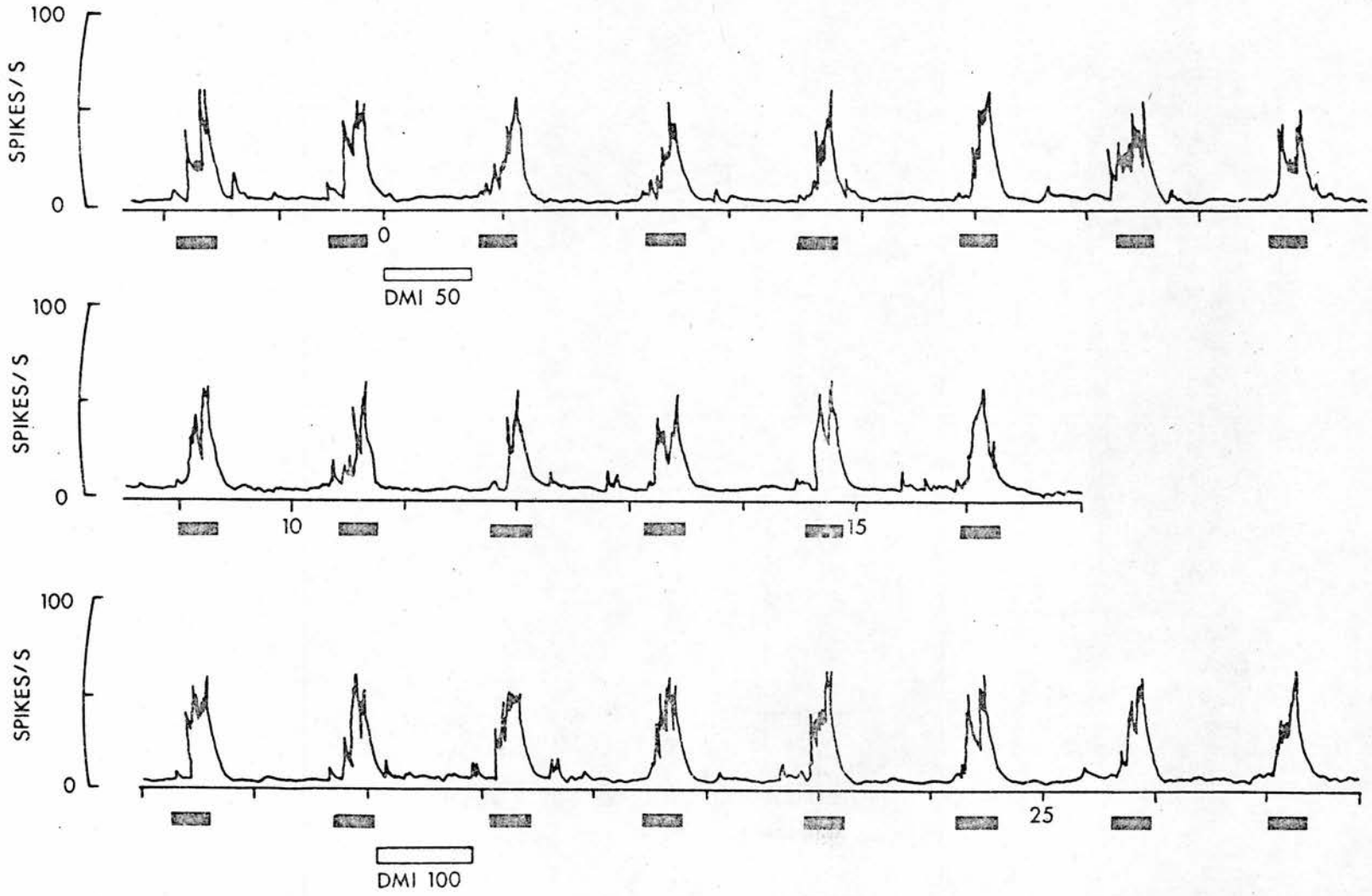

A POTENTIATION
MAX. INCREASE IN SIZE OF RESPONSE (PERCENT)
200
150
100
50
0
20%
+
−
DOPAMINE
+
−
NORADRENALINE
+
5-HT
+
ACh
+
GLU
B ANTAGONISM
MAX. DECREASE IN SIZE OF RESPONSE (PERCENT)
100
50
0
20%
+
−
DOPAMINE
+
−
NORADRENALINE
+
5-HT
+
ACh
+
GLU

FIG. 21. Effect of desipramine (DMI) on responses of a single caudate neurone to glutamate. Excerpts from the ratemeter recording of the firing rate of the neurone. Ordinates: firing rate (spikes/s); absciassae: time (minutes). Solid bars underneath the abscissae indicate the applications of glutamate (50 nA). Open bars underneath the abscissae indicate the applications of desipramine. Desipramine, when ejected with current of 50 nA and 100 nA, had no effect on the size of responses to glutamate.


100
SPIKES/S
0
0
DMI 50
100
SPIKES/S
0
10
15
100
SPIKES/S
0
25
DMI 100

desipramine, and this might counteract the anti-Parkinsonian effect of desipramine. This may explain why a small dose of a potent anticholinergic drug has to be administered concurrently with desipramine in order to obtain the full therapeutic benefit from the antidepressant in Parkinsonism (Yahr & Duvoisin, 1972).

3:3 THE EFFECTS OF IPRINDOLE ON RESPONSES OF SINGLE CORTICAL AND CAUDATE NEURONES TO MONOAMINES AND ACETYLCHOLINE

3:3.1 INTRODUCTION

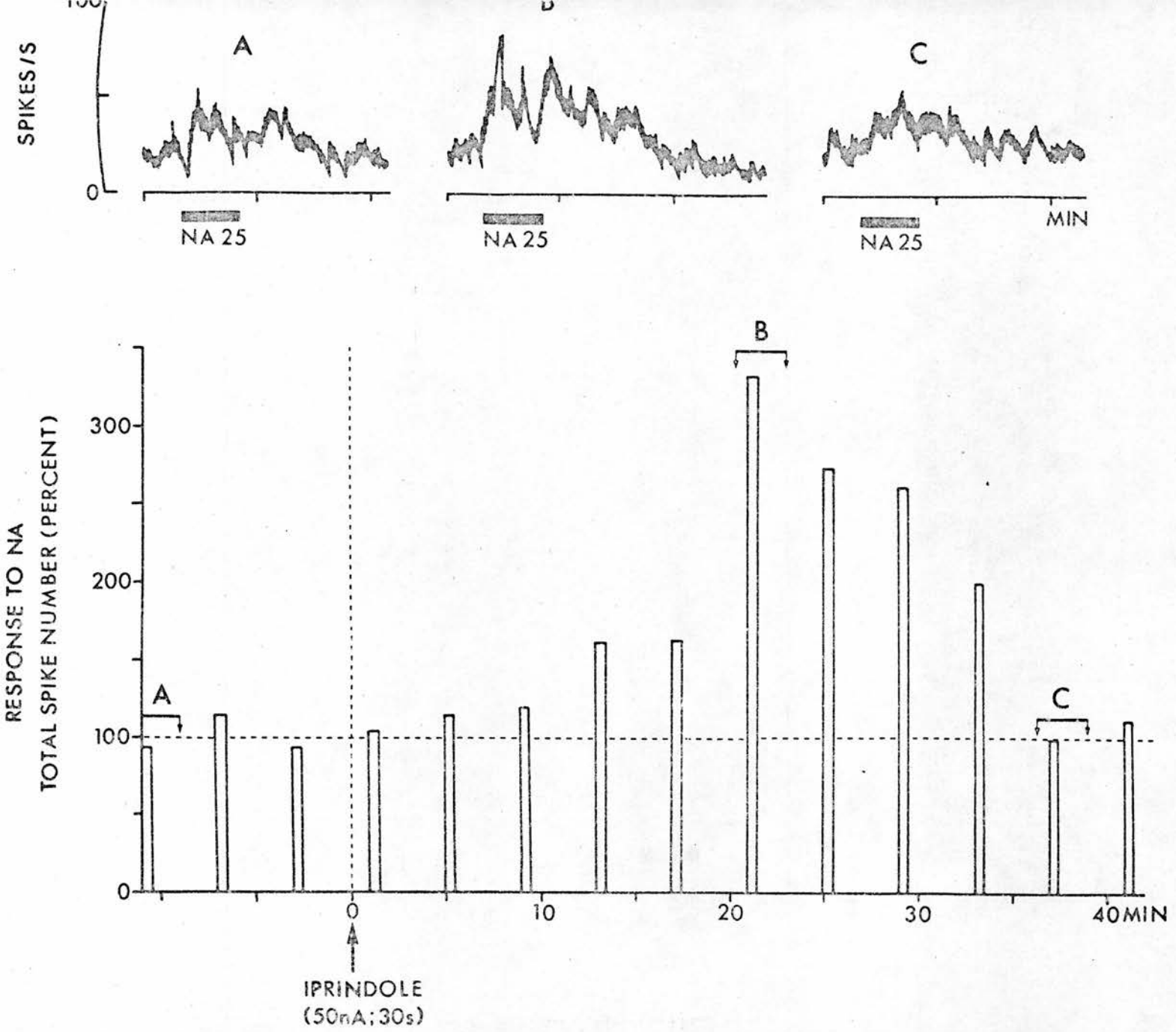
Iprindole is a tricyclic antidepressant drug with a similar structure and clinical antidepressant efficacy to imipramine (Imlah, Murphy & Mellor, 1968; Rickels, Chung, Csanalosi, Sablosky & Simon, 1973). Similarly to imipramine, iprindole potentiates the peripheral effects of NA (Gluckman & Baum, 1969). However, in contrast to imipramine and other tricyclic antidepressant compounds, iprindole does not block the uptake of NA into sympathetically innervated tissues (Gluckman & Baum, 1969; Lahti & Maickel, 1971). It has also been reported that, in contrast to imipramine and desipramine, iprindole is ineffective in blocking the uptake of NA (Ross et al, 1971; Rosloff & Davis, 1974), dopamine (DA), and 5HT (Ross et al, 1971) into brain tissue. It is not known, however, how pharmacological responses to the monoamines are affected by iprindole in the brain. In the experiments reported below, the technique of microelectrophoresis was used in order to investigate how responses of single cortical and caudate neurones to NA, DA, 5HT and ACh could be modified by iprindole.

CEREBRAL CORTEX

3:3.2 EFFECT OF IPRINDOLE ON NEURONAL FIRING

The effect of iprindole on the firing rate was studied on 68 cells. The dose of antidepressant applied was 30 - 100 nA applied for 30 - 60 seconds. On 4 cells

FIG. 22. Potentiation of excitatory responses of a single cortical neurone to noradrenaline (NA) by iprindole. Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to NA. (B) Potentiated response to NA 21 minutes after a brief application of iprindole (50 nA; 30 seconds). (C) Recovery of control response 37 minutes after the application of iprindole. The graph at the bottom shows the time-course of the entire study (as in Fig. 13).


SPIKES/S
0
A
B
C
NA 25
NA 25
NA 25
MIN
RESPONSE TO NA
TOTAL SPIKE NUMBER (PERCENT)
300
200
100
0
A
B
C
0
10
20
30
40MIN
IPRINDOLE
(50nA; 30s)

(6%) the firing rate was increased during the application of iprindole, whereas on 19 cells (28%) the firing rate was decreased. There was no significant correlation between the dose of iprindole applied and the effect on neuronal firing. An example of both the excitatory and depressant effects of iprindole on the firing rate is shown in Figure 12B. On occasions, a reduction in spike amplitude was observed; such cells were not used for drug interaction studies.

3:3.3 EFFECT OF IPRINDOLE ON RESPONSES TO NORADRENALINE

3:3.3.1. Excitatory responses

Both potentiation and antagonism of the responses to NA could be observed after a brief application (30 - 100 nA for 30 - 60 seconds) of iprindole.

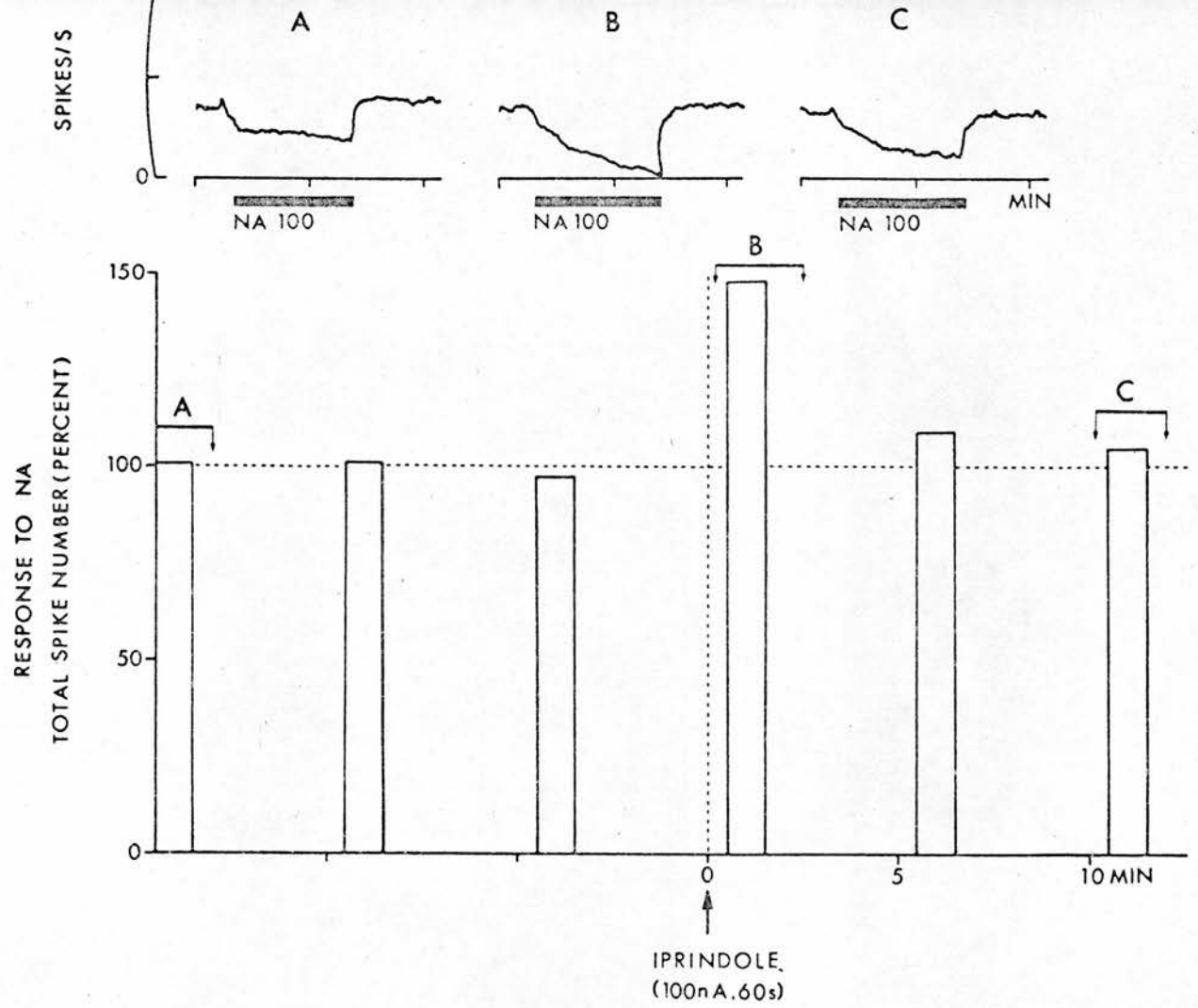
Potentiation was seen on 6 cells. An example of potentiation is shown in Figure 21. On one cell, the response was first reduced in size following the application of iprindole; this antagonism was followed later by potentiation, and finally by recovery of the control response. On 2 cells no significant change in the size of the response could be observed after the application of iprindole. The degrees of potentiation and antagonism observed on each cell are summarised in Figure 28.

3:3.3.2. Depressant responses

Both potentiation and antagonism of the depressant responses could be observed after a brief application of iprindole.

Potentiation was seen on 10 cells, antagonism was seen on 3 cells. On 2 cells both antagonism and

FIG. 23. Potentiation of depressant responses of a single cortical neurone to noradrenaline (NA) by iprindole. Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to NA. (B) Potentiated response one minute after a brief application of iprindole (100 nA; 60 seconds). (C) Recovery of the control response 11 minutes after the application of iprindole Graph at the bottom shows the time-course of the entire study (as in Fig. 13)


SPIKES/S
0
A
B
C
NA 100
NA 100
NA 100
MIN
RESPONSE TO NA
TOTAL SPIKE NUMBER (PERCENT)
150
100
50
0
A
B
C
0
5
10 MIN
IPRINDOLE
(100nA.60s)

potentiation could be observed; in both cases antagonism preceded potentiation. An example of the potentiation of the depressant response to NA by iprindole is shown in Figure 23.

On one cell no significant change in the size of response could be observed after the application of iprindole. The degrees of potentiation and antagonism seen in each cell are summarised in Figure 28.

3:3.4 EFFECT OF IPRINDOLE ON RESPONSES TO DOPAMINE

3:3.4.1. Excitatory responses

Both potentiation and antagonism of excitatory responses to DA could be observed after a brief application of iprindole.

Potentiation of the responses was seen on 5 cells, antagonism was seen on 4 cells. On 3 cells both antagonism and potentiation of the response could be observed; antagonism always preceded potentiation. Figure 24 shows an example of potentiation, and Figure 25 shows an example of antagonism followed by potentiation. On one cell no significant change in the size of responses could be observed after the application of iprindole. The degrees of potentiation or antagonism seen in each cell are summarised in Figure 28.

3:3.4.2. Depressant responses

Potentiation was seen on 3 cells depressed by DA, antagonism was seen on one cell. Figure 28 summarises the degrees of potentiation or antagonism observed in each cell.

FIG. 24. Potentiation of excitatory responses of a single cortical neurone to dopamine (DA) by iprindole. Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to DA. (B) Potentiated response to DA 17 minutes after a brief application of iprindole (100 nA; 60 seconds). (C) Recovery of the control response 31 minutes after the application of iprindole. Graph at the bottom shows the time-course of the entire study (as in Fig. 13).

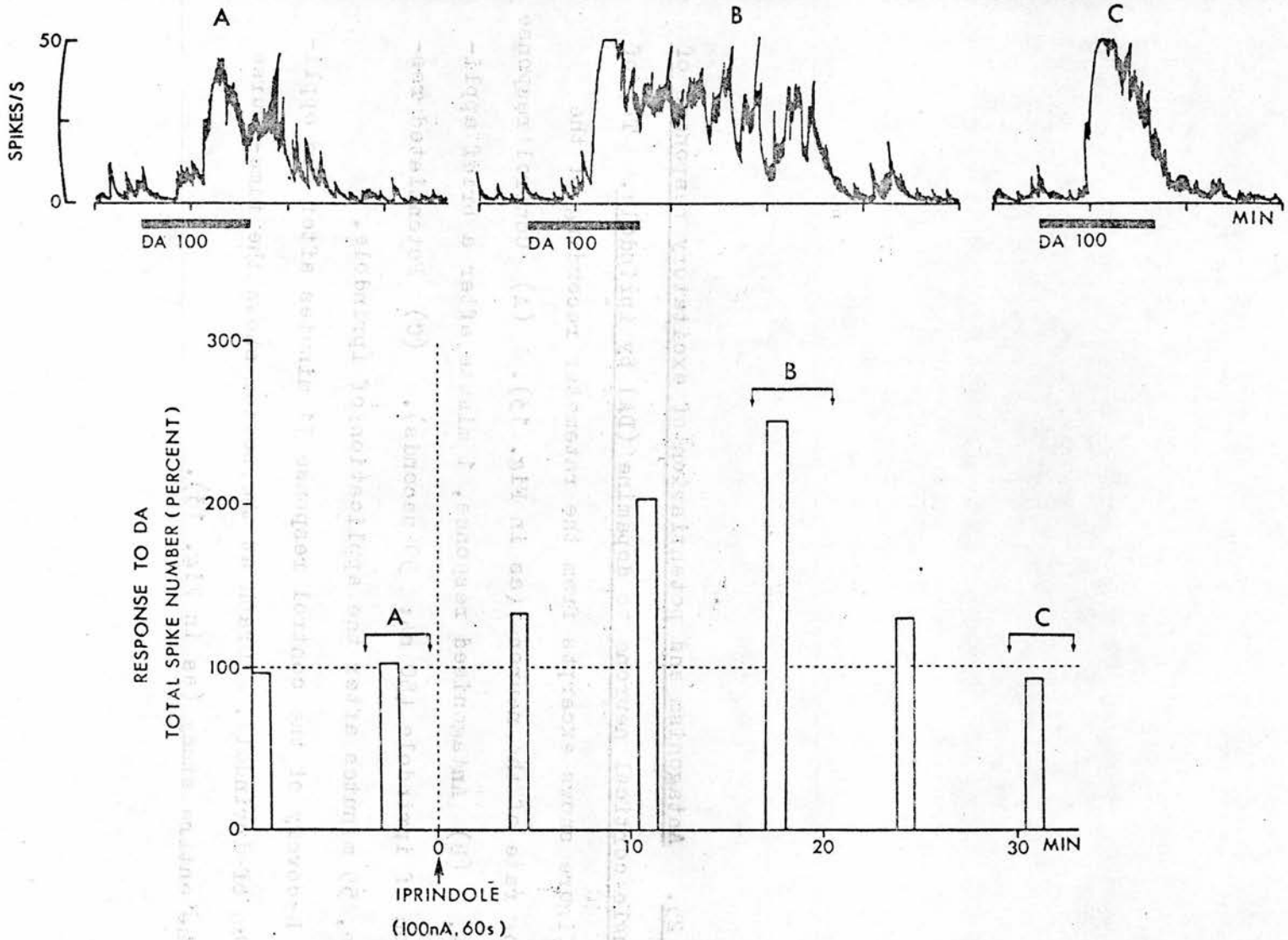
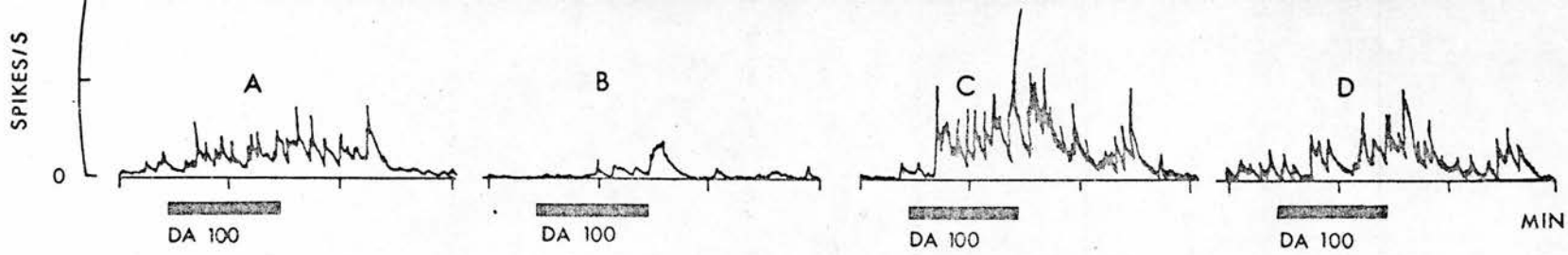
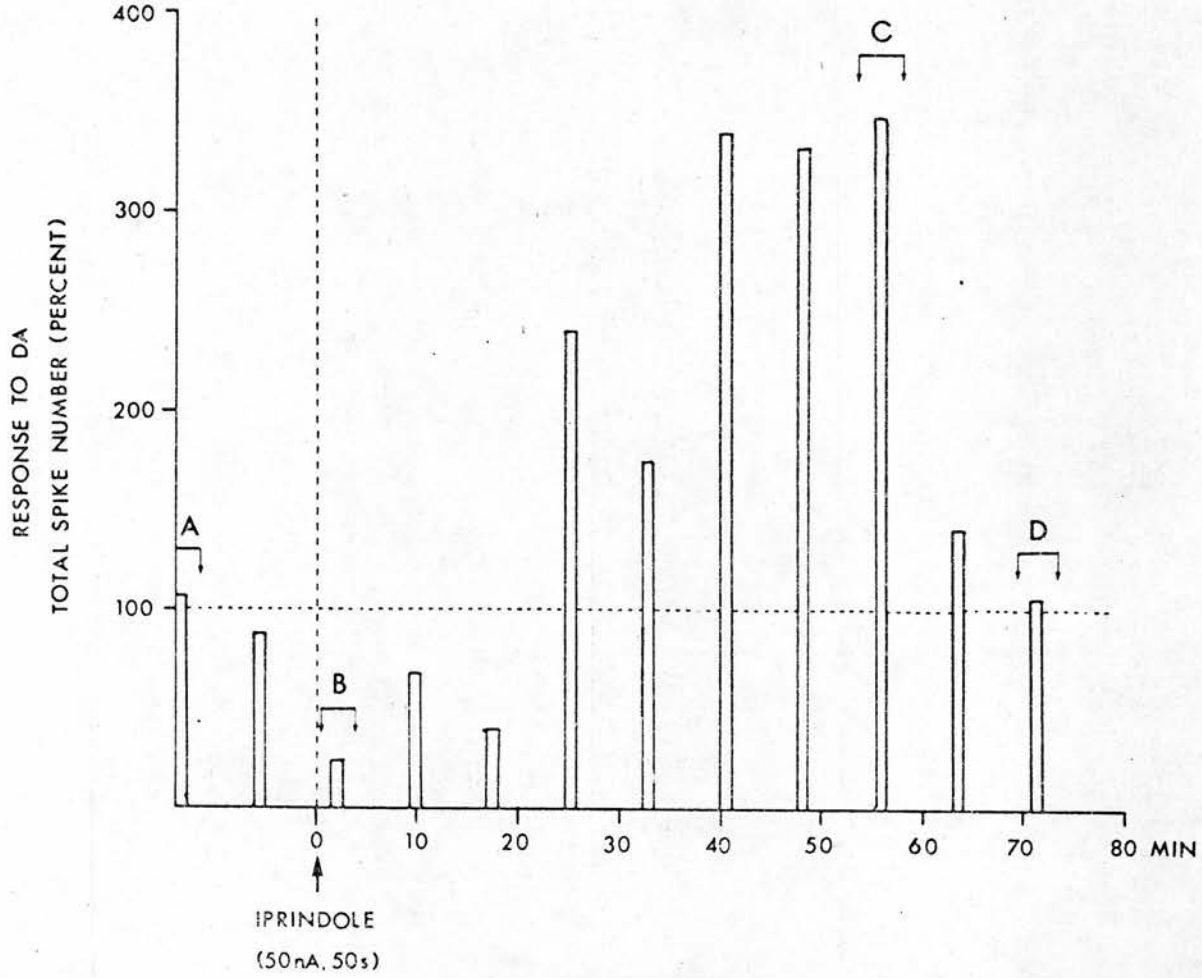

A
B
C
50
SPIKES/S
0
DA 100
DA 100
DA 100
MIN
300
200
100
0
RESPONSE TO DA
TOTAL SPIKE NUMBER (PERCENT)
A
B
C
0
10
20
30 MIN
IPRINDOLE
(100nA, 60s)

FIG. 25. Antagonism and potentiation of excitatory responses of a single cortical neurone to dopamine (DA) by iprindole. Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to DA. (B) Antagonised response, 1 minute after a brief application of iprindole (50 nA; 50 seconds). (C) Potentiated response, 55 minutes after the application of iprindole. (D) Recovery of the control response 71 minutes after the application of iprindole. Graph at the bottom shows the time-course of the entire study (as in Fig. 13).


SPIKES/S
0
A
B
C
D
DA 100
DA 100
DA 100
DA 100
MIN


400
300
200
100
RESPONSE TO DA
TOTAL SPIKE NUMBER (PERCENT)
A
B
C
D
0
10
20
30
40
50
60
70
80 MIN
IPRINDOLE
(50nA, 50s)

3:3.5 EFFECT OF IPRINDOLE ON RESPONSES TO ACETYLCHOLINE

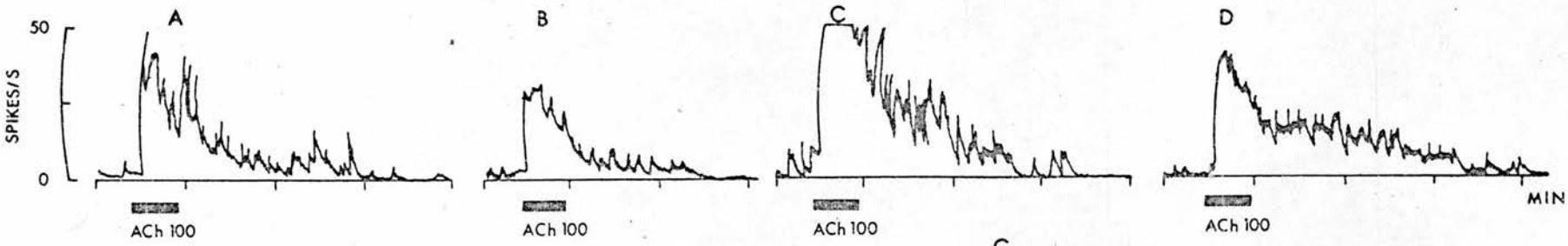
Both potentiation and antagonism of excitatory responses to ACh could be observed after a brief application of iprindole. Potentiation of the response was seen on 3 cells, antagonism was seen on 5 cells. On 3 cells, both antagonism and potentiation could be observed; antagonism always preceded potentiation. An example of antagonism followed by potentiation is shown in Figure 26. On 2 cells iprindole had no significant effect on the size of the responses to ACh. The data are summarised in Figure 28.

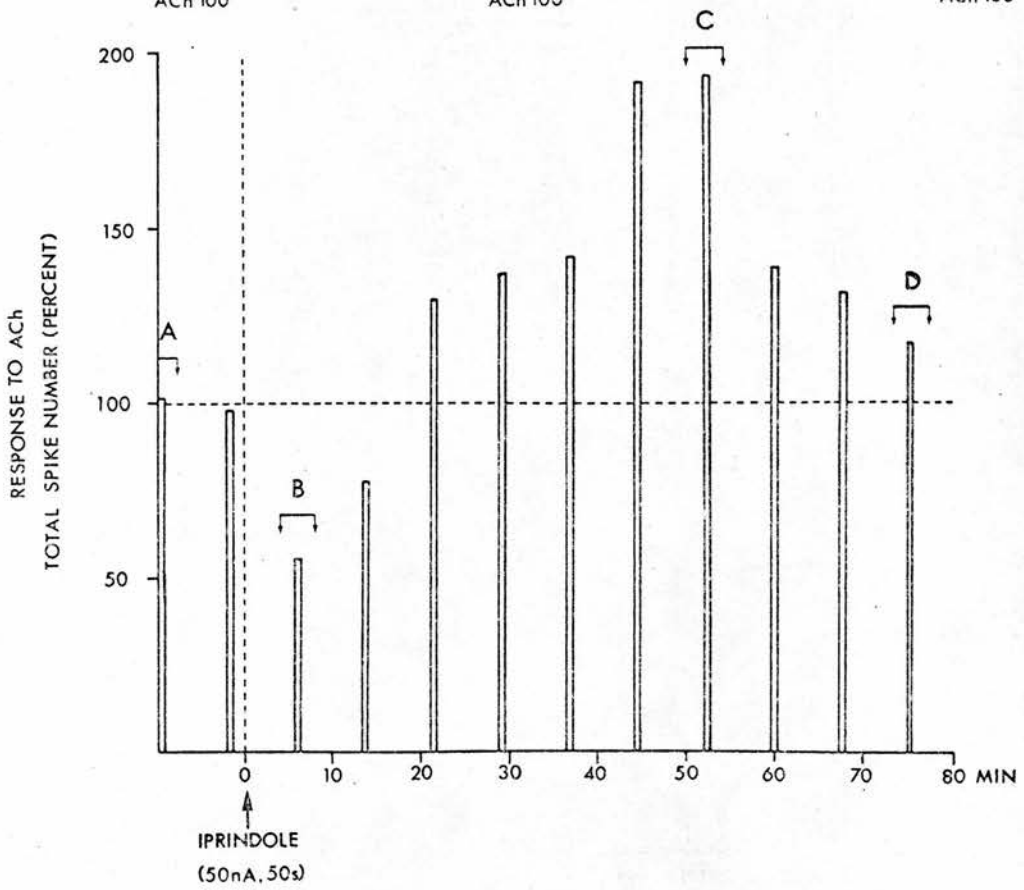
On one cell, the effect of the continuous application of iprindole was examined (Figure 27). Iprindole, when applied with a current of 5 nA, potentiated the response to ACh. When the current used to apply iprindole was increased to 10 nA, the size of the response to ACh was attenuated back to its control level. When an even larger current was used to apply iprindole (15 nA), the response to ACh was antagonised. After the application of iprindole had been terminated, the response recovered from the antagonism; this was followed by the potentiation of the response and finally by recovery of the control response.

3:3.6 EFFECT OF IPRINDOLE ON RESPONSES TO GLUTAMATE

The effect of iprindole on excitatory responses to glutamate was studied on 10 cells. The effects of glutamate on the firing rate were studied for 20 - 30 minutes following the application of iprindole. On none of these

FIG. 26. Antagonism and potentiation of excitatory responses of a single cortical neurone to acetylcholine (ACh) by iprindole. Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to ACh. (B) Antagonised response, 6 minutes after a brief application of iprindole (50 nA; 50 seconds). (C) Potentiated response, 52 minutes after the application of iprindole. (D) Recovery of the control response 75 minutes after the application of iprindole. Graph at the bottom shows the time-course of the entire study (as in Fig. 13).


SPIKES/S
50
0
A
B
C
D
ACh 100
ACh 100
ACh 100
ACh 100
MIN


RESPONSE TO ACh
TOTAL SPIKE NUMBER (PERCENT)
200
150
100
50
A
B
C
D
0
10
20
30
40
50
60
70
80 MIN
IPRINDOLE
(50nA, 50s)

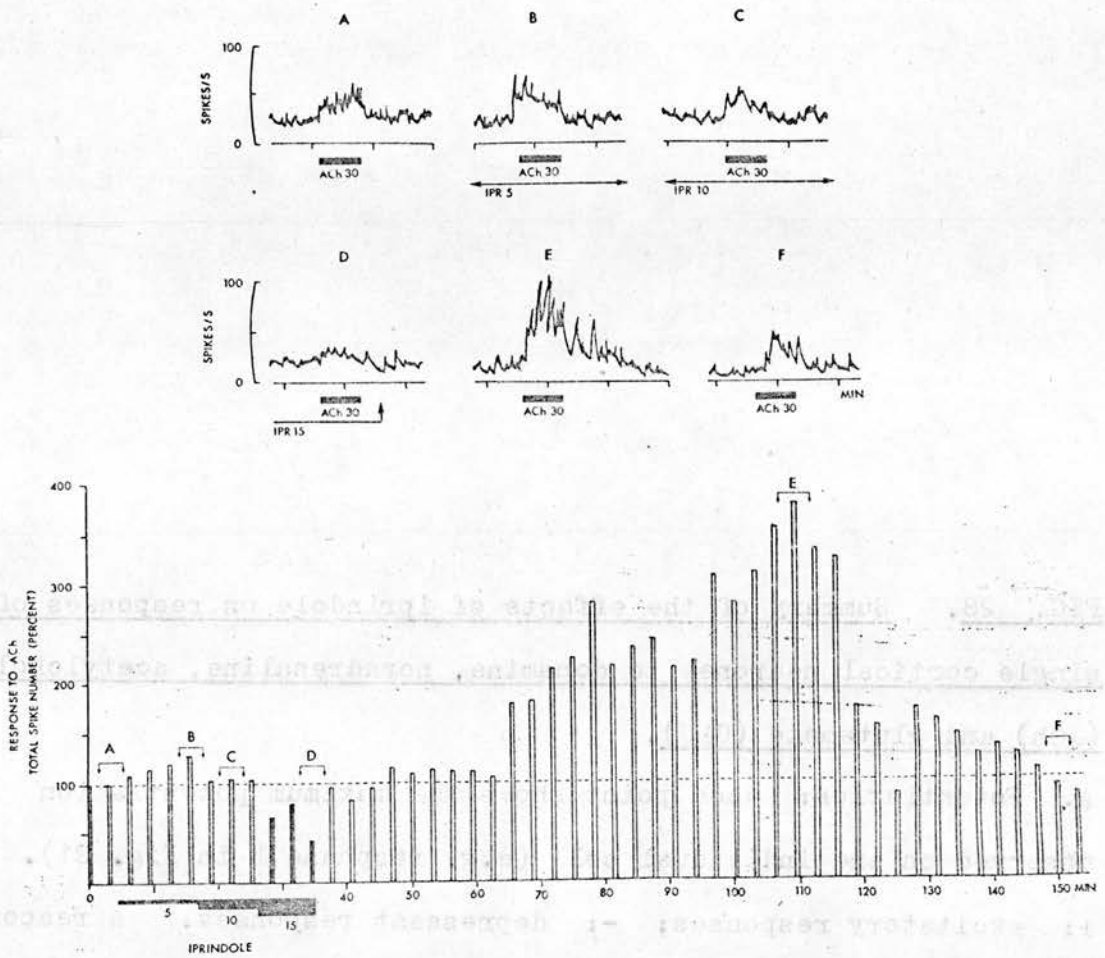
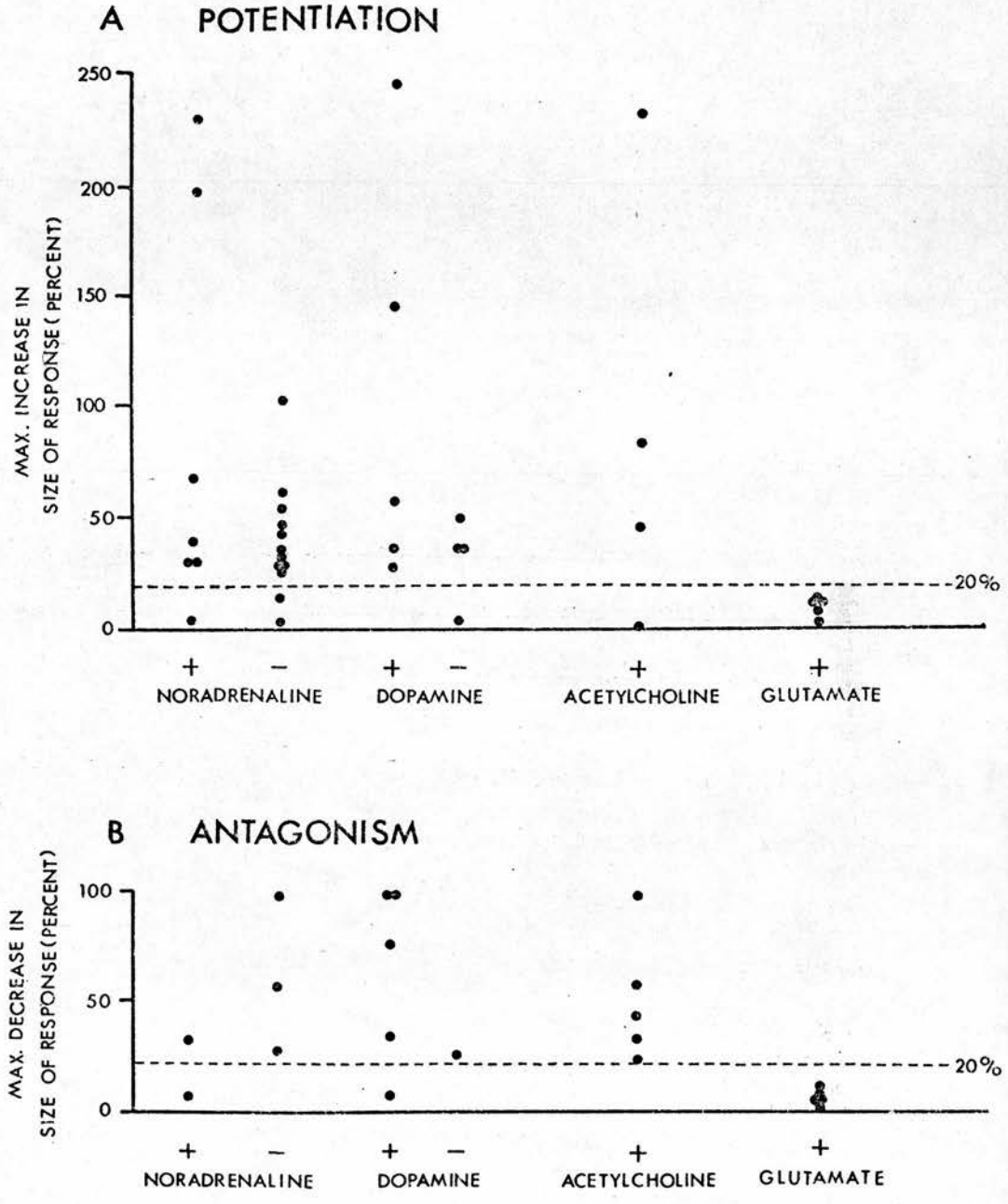


FIG. 27. Antagonism and potentiation of excitatory responses of a single cortical neurone to acetylcholine (ACh) by iprindole (IPR). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to ACh. (B) Response to ACh was potentiated during the application of iprindole (5 nA). (C) Response to ACh was attenuated back to the control size during the application of iprindole (10 nA). (D) Response to ACh was antagonised in the presence of iprindole (15 nA). (E) Response to ACh was potentiated 74 minutes after the application of iprindole had been terminated. (F) Recovery of response to ACh 115 minutes after the application of iprindole had been terminated. The graph at the bottom shows the time-course of the entire study (as in Fig. 13). The horizontal bars under the abscissa refer to the intensity of the current used to apply iprindole.

FIG. 28. Summary of the effects of iprindole on responses of single cortical neurones to dopamine, noradrenaline, acetylcholine (ACh) and glutamate (GLU).

A. Potentiation: each point shows the maximum potentiation observed on one individual cell (e.g. response B in Fig. 21). +: excitatory responses; -: depressant responses. A response was regarded as potentiated if there was more then 20% increase over the size of the control response (see 2:5.4).

B. Antagonism: each point shows the maximum degree of antagonism observed on one individual cell (e.g. response B in Fig. 24). A response was regarded as antagonised if there was more than 20% decrease over the size of the control response (see 2:5.4).


A POTENTIATION
MAX. INCREASE IN SIZE OF RESPONSE (PERCENT)
250
200
150
100
50
0
20%
+
−
+
−
+
+
NORADRENALINE
DOPAMINE
ACETYLCHOLINE
GLUTAMATE
B ANTAGONISM
MAX. DECREASE IN SIZE OF RESPONSE (PERCENT)
100
50
0
20%
+
−
+
−
+
+
NORADRENALINE
DOPAMINE
ACETYLCHOLINE
GLUTAMATE

cells could any significant change be observed in the size of the responses to glutamate after the application of iprindole (see Figure 28). An example of a study of the effect of iprindole on responses of a single cortical neurone to glutamate is shown in Figure 29.

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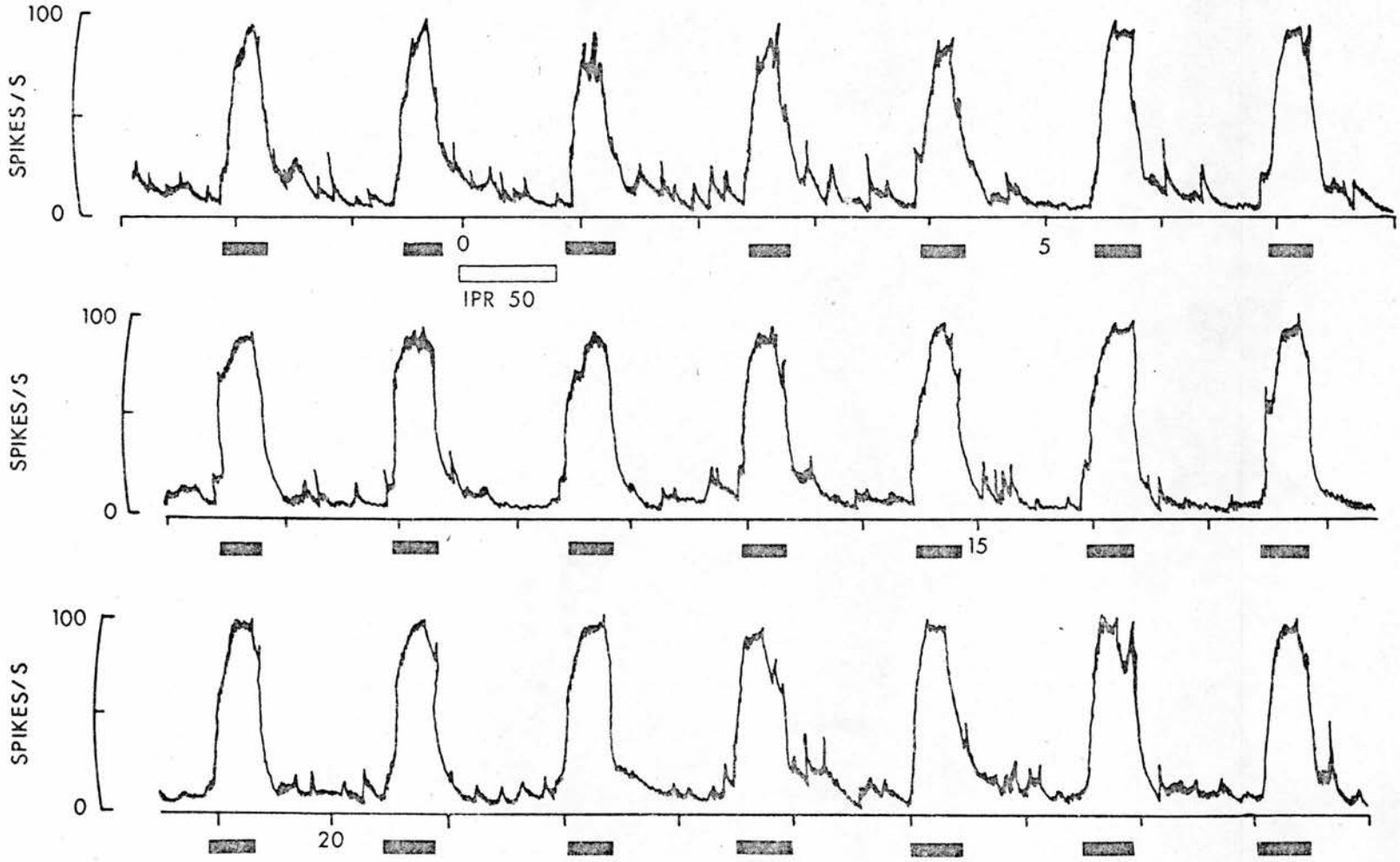
3:3.7 THE EFFECTS OF IPRINDOLE ON RESPONSES TO NORADRENALINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE

The direct effect of iprindole on neuronal firing in the caudate nucleus was studied on 18 cells: 3 cells were excited, 2 cells were depressed, whereas on 13 cells there was no change in the firing rate. The effect of iprindole was also examined on neuronal responses to NA, DA and 5HT. The same patterns of drug interaction could be observed as described above. Following a brief application of iprindole, both antagonism and potentiation of excitatory responses to NA (2 cells), DA (3 cells) and 5HT (3 cells) could be observed. Similarly, both antagonism and potentiation of depressant responses to NA (3 cells) and DA (5 cells) was seen. Excitatory responses to glutamate (3 cells) were not affected by iprindole. Figure 30 shows the dual effect of iprindole on excitatory responses to 5HT, and Figure 31 shows an example of the potentiation of depressant responses to DA by iprindole.

3:3.8 DISCUSSION

A brief application of iprindole did not affect the firing rate of the majority of neurones tested in the cortex and in the caudate nucleus. On a small number of cells,

FIG. 29. Effect of iprindole (IPR) on responses of a single cortical neurone to glutamate. Excerpts from the ratemeter recording of the firing rate of the neurone. Ordinates: firing rate (spikes/s); abscissae: time (minutes). Solid bars underneath the abscissae indicate the applications of glutamate (50 nA); the open bar indicates the application of iprindole (50 nA). Iprindole had no effect on the size of excitatory responses to glutamate.

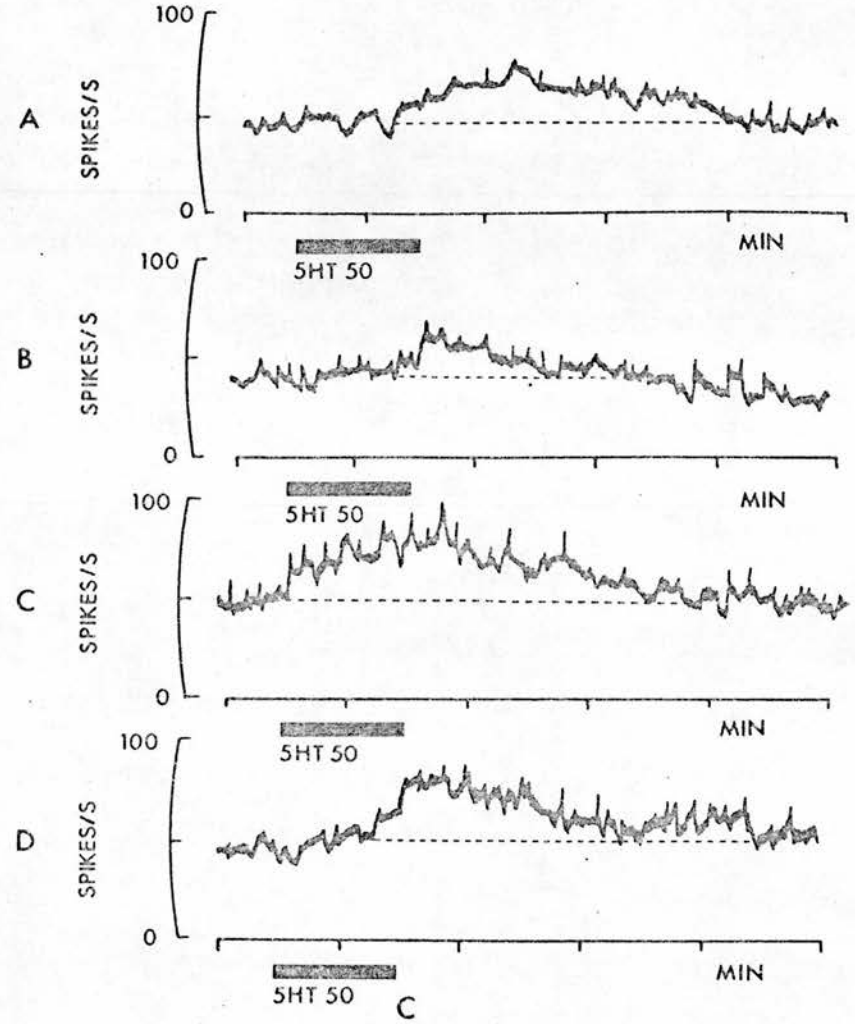

100
SPIKES/S
0
0
5
IPR 50
100
SPIKES/S
0
15
100
SPIKES/S
0
20

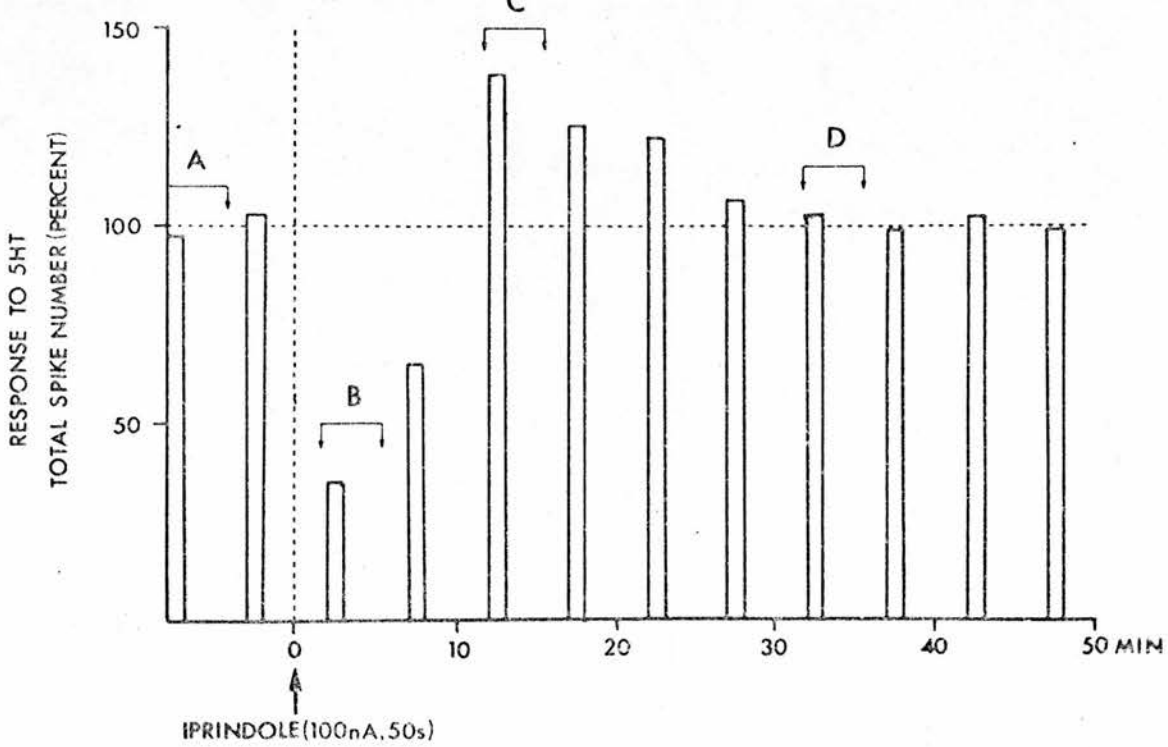
however, a response to iprindole was observed. A possible explanation for this effect of iprindole itself could be that it reflects the interaction between engogenously released monoamine transmitters and iprindole. As responses to ACh are also affected by iprindole, an interaction with ACh released by cholinergic inputs to the neurone should also be considered. Similarly to iprindole, imipramine and desipramine can also evoke excitatory and depressant responses on spontaneously firing cortical neurones (Bradshaw et al, 1974). On a few cells, a reduction in spike amplitude was observed in response to iprindole. This probably reflects the local anaesthetic action of tricyclic antidepressant drugs (Domenjoz & Theobald, 1959).

Iprindole has a dual effect on responses of cortical and caudate neurones to monoamines (NA and DA in the cerebral cortex, NA, and DA and 5HT in the caudate nucleus). Both antagonism and potentiation of the response to a monoamine could be observed after a brief application of iprindole. When both antagonism and potentiation of the response occurred after a single application of iprindole, antagonism invariably preceded the development of potentiation (see Figure 25). As after a brief ejecting pulse, the concentration of iprindole probably rises quickly to a peak, and then gradually declines (Bradshaw et al, 1974), antagonism of the responses to the monoamine is likely to reflect a higher, whereas potentiation a lower concentration of the antidepressant (see Bradshaw et al, 1974).

FIG. 30 Antagonism and potentiation of excitatory responses a single caudate neurone to 5-hydroxytryptamine (5HT) by iprin

Top of the figure shows excerpts from the ratemeter recording the firing rate of the neurone (as in Fig. 13). (A) Control response to 5HT. (B) Antagonised response, 3 minutes after brief application of iprindole (100 nA; 50 seconds). (C) Potentiated response, 12 minutes after the application of iprindole. (D) Recovery of the control response 32 minutes after the application of iprindole. Graph at the bottom show the time-course of the entire study (as in Fig. 13).


A
B
C
D
100
0
SPIKES/S
MIN
5HT 50

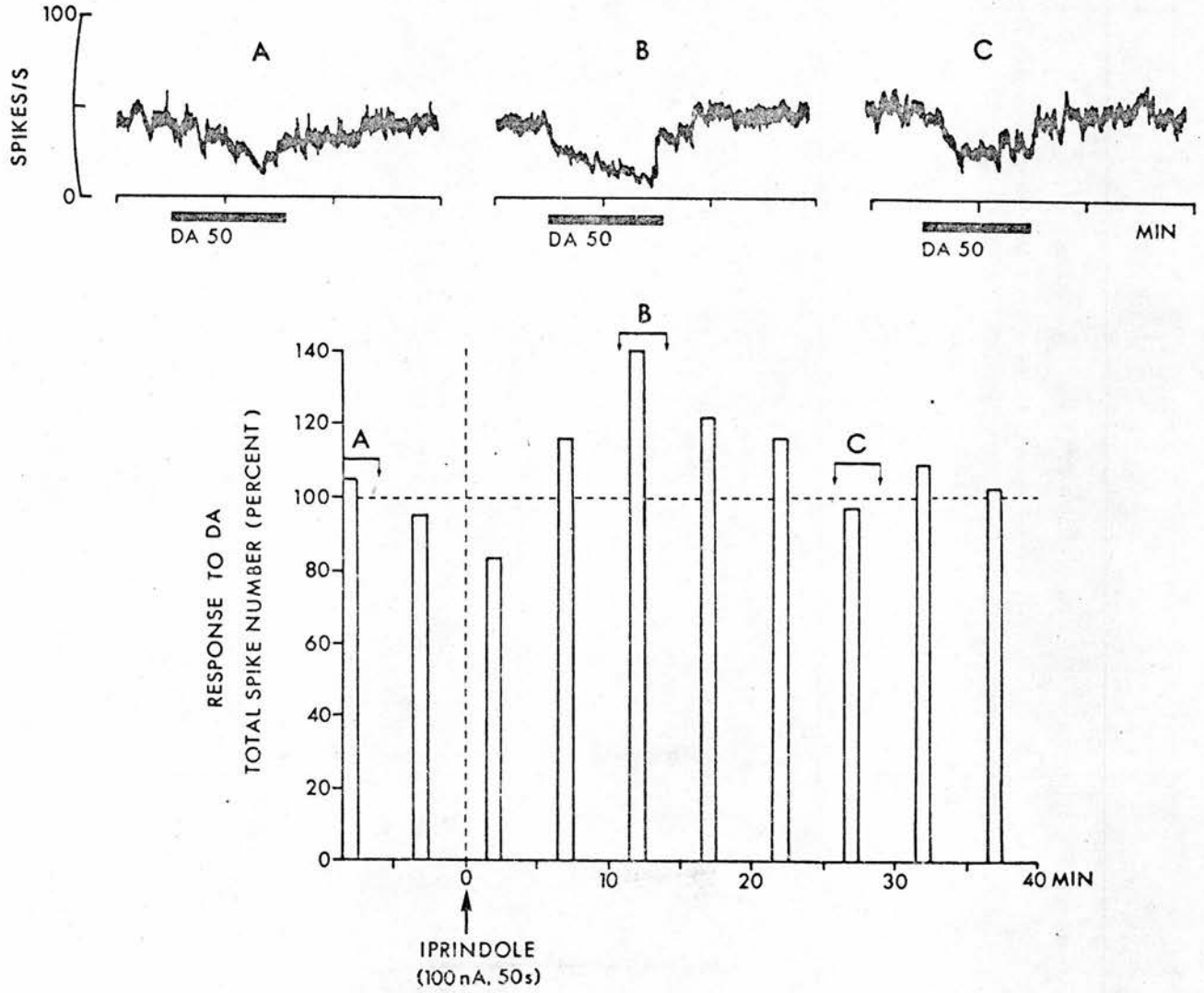

RESPONSE TO 5HT
TOTAL SPIKE NUMBER (PERCENT)
150
100
50
A
B
C
D
0
10
20
30
40
50 MIN
IPRINDOLE (100nA, 50s)

The most plausible explanation for antagonism is the blockade of monoamine receptors on the post-synaptic neurone. This would indicate that iprindole probably shares the α-adrenoceptor blocking and antiserotonin actions of other tricyclic antidepressants (Domenjoz & Theobald, 1959; Callingham, 1967).

As iprindole is ineffective in blocking the uptake of NA, DA and 5HT into the brain tissue (Ross et al, 1971), uptake blockade cannot be an explanation for the potentiation observed in these experiments. An alternative explanation for potentiation will be discussed in Chapter 4.

Iprindole has a dual effect not only on responses to monoamines, but also on responses to ACh: both antagonism and potentiation can be observed. It is likely that the antagonism reflects a higher, whereas potentiation a lower concentration of iprindole (see Figure 27, and Bevan et al, 1975a). This could explain the results obtained from the study in which iprindole was applied continuously (see Figure 27). When iprindole was ejected continuously from the micropipette by a low current (5 nA), the excitatory response to ACh was potentiated. When a larger current (15 nA) was used to apply iprindole, the response to ACh was antagonised. After the application of iprindole had been terminated, the concentration of iprindole probably declined slowly (see above) so that potentiation of the response to ACh gradually developed, to be followed later by recovery of the control response. As in the case of imipramine, desipramine and atropine (Bevan et al, 1975a), the antagonism can be interpreted by a blockade of muscarinic receptors, since it has been reported that iprindole blocks the effects of ACh in peripheral test systems (Gluckman & Baum, 1969). The

FIG. 31. Potentiation of depressant responses of a single caudate neurone to dopamine (DA) by iprindole. Top of the f shows excerpts from the ratemeter recording of the firing rat the neurone (as in Fig. 13). (A) Control response to DA. (B) Potentiated response to DA 12 minutes after a brief appl cation of iprindole (100 nA; 50 seconds). (C) Recovery of control response 27 minutes after the application of iprindol Graph at the bottom shows the time-course of the entire study (as in Fig. 13).


100
SPIKES/S
0
A
B
C
DA 50
DA 50
DA 50
MIN
B
140
120
A
C
100
80
60
40
20
0
RESPONSE TO DA
TOTAL SPIKE NUMBER (PERCENT)
0
10
20
30
40 MIN
IPRINDOLE
(100 nA, 50s)

potentiation of neuronal responses to ACh by iprindole may be due to the selective blockade of masked inhibitory receptors (see Bevan et al, 1975a and Chapter 4).

3:4 THE EFFECT OF DESIPRAMINE ON RESPONSES OF SINGLE CORTICAL NEURONES TO MESCALINE

3:4.1 INTRODUCTION

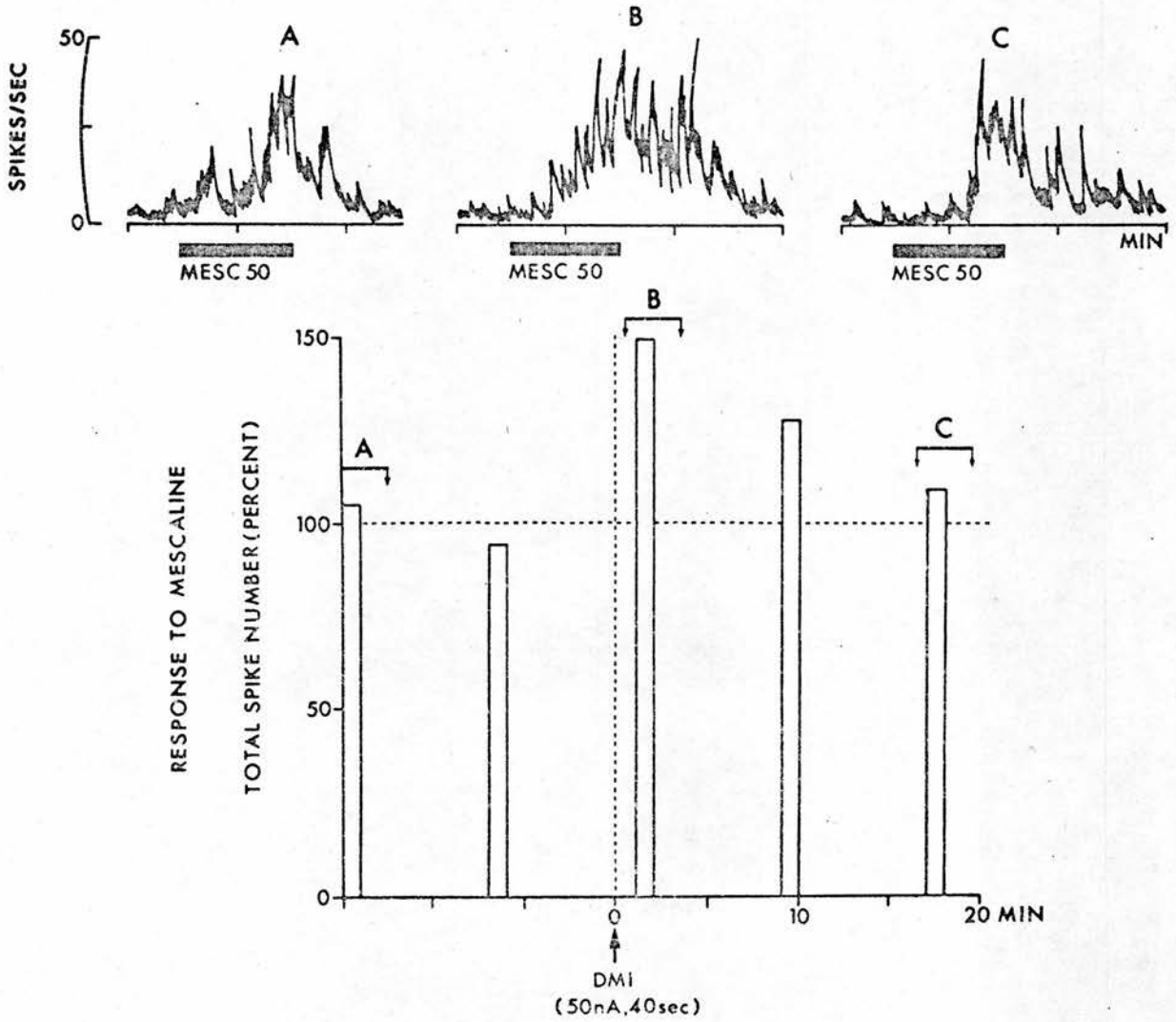
The uptake blockade hypothesis of potentiation suggests that the potentiation of monoamine responses by tricyclic antidepressant drugs is a consequence of the ability of such drugs to inhibit the accumulation of monoamines into nerve terminals. Iversen (1967) has shown that mescaline (3,4,5-trimethoxyphenylethylamine) has a very low affinity for catecholamine uptake mechanisms in the periphery. It was therefore of great interest to determine whether neuronal responses to mescaline could be potentiated by tricyclic antidepressant drugs, since it is unlikely that such an effect would be mediated by an effect on the uptake of mescaline.

When applied to single cortical neurones, mescaline, like NA and 5HT, evoked both excitatory and depressant responses (Bradshaw et al, 1971b; Bevan et al, 1974a; 1974b). It has also been shown that responses to NA, 5HT and mescaline can be antagonised by methysergide and sotalol, while responses to ACh are not affected (Bevan et al, 1974a; 1974b). In the present study, the effect of desipramine on responses of single cortical neurones to mescaline was investigated.

3:4.2 EFFECT OF DESIPRAMINE ON RESPONSES TO MESCALINE

Both potentiation and antagonism of excitatory responses to mescaline could be observed after a brief application (30 - 100 nA for 30 - 60 seconds) of desipramine.

FIG. 32. Potentiation of excitatory responses of a single cortical neurone to mescaline (MESC) by desipramine (DMI). Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control response to mescaline. (B) Potentiated response to mescaline one minute after a brief application of DMI (50 nA; 40 seconds). (C) Recovery of control response 17 minutes after the application of desipramine. The graph at the bottom shows the time-course of the entire study (as in Fig. 13).


SPIKES/SEC
50
0
A
B
C
MESC 50
MESC 50
MESC 50
MIN
RESPONSE TO MESCALINE
TOTAL SPIKE NUMBER (PERCENT)
150
100
50
0
A
B
C
0
10
20 MIN
DMI
(50nA, 40sec)

Potentiation of the response was seen on 10 cells. An example of potentiation is shown in Figure 32. Antagonism of the response to mescaline was seen on 5 cells. On 5 cells, both potentiation and antagonism could be observed; antagonism always preceded potentiation. On 2 cells, no significant change in the size of the response to mescaline could be observed after the application of desipramine.

3:4.3 EFFECT OF DESIPRAMINE ON RESPONSES TO GLUTAMATE

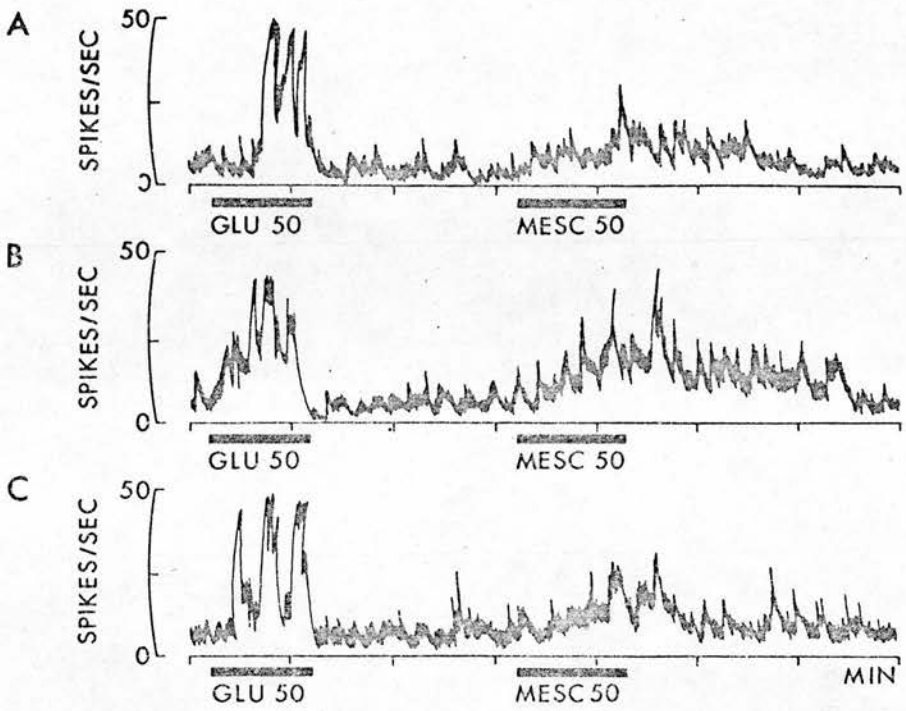
The effect of desipramine on the excitatory responses to glutamate was studied on 10 cortical neurones. The effects of glutamate on the firing rate were studied for 20 - 30 minutes following the application of desipramine. On none of these cells could any significant change be observed in the size of the response to glutamate after the application of desipramine (see Figure 33).

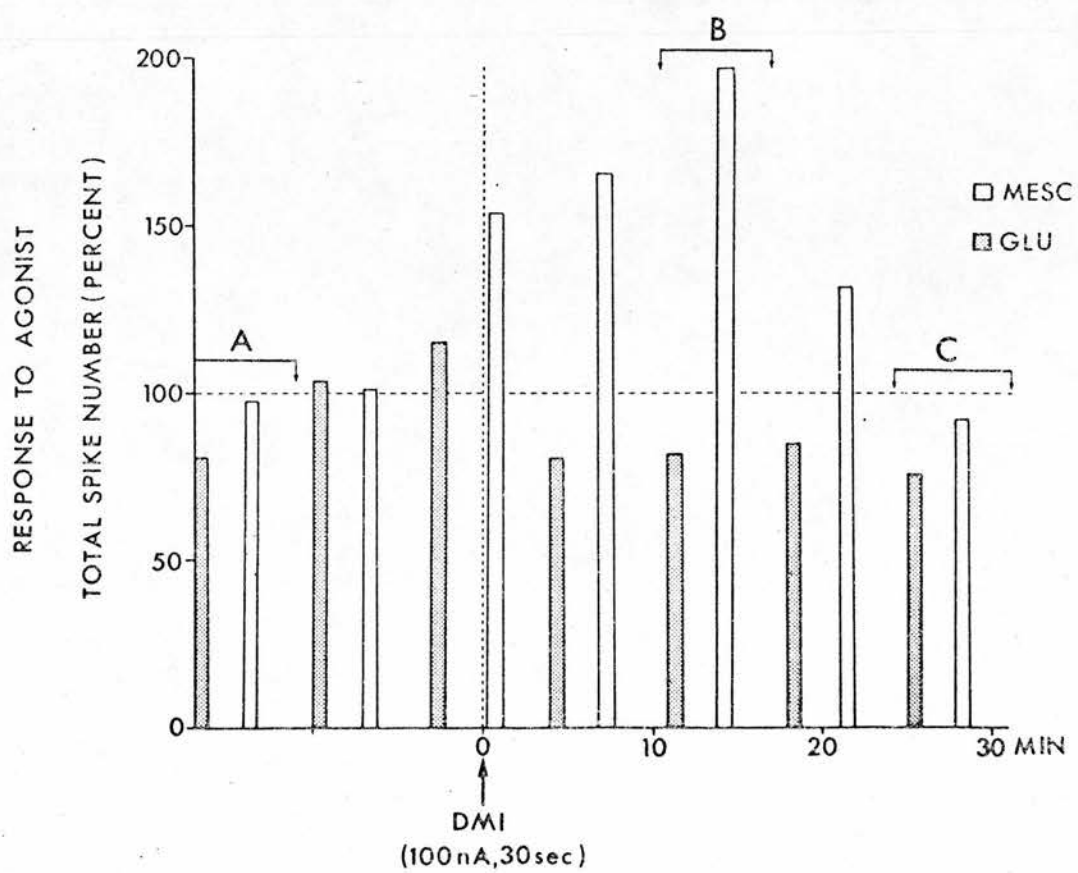
3:4.4 DISCUSSION

The results show that desipramine had a dual effect on the responses of single cortical neurones to mescaline; both antagonism and potentiation of the responses could be observed. Responses to glutamate were not affected by desipramine. Thus responses to all three monoamines, NA, 5HT and mescaline, are affected in a similar way by desipramine.

As receptors to mescaline, NA and 5HT are likely to be very similar on cortical neurones (Bevan et al, 1974a), the antagonism of responses to mescaline could be interpreted on the basis of the α-adrenoceptor blocking effect of desipramine (Turker & Khairallah, 1967), or alternatively, by the

FIG. 33. Effect of desipramine (DMI) on excitatory responses to mescaline (MESC) and to glutamate (GLU) of a single cortical neurone. Top of the figure shows excerpts from the ratemeter recording of the firing rate of the neurone (as in Fig. 13). (A) Control responses to mescaline and glutamate. (B) Potentiation of the response to mescaline 14 minutes after the application of DMI (100 nA; 30 seconds). The response to glutamate was not affected. (C) Recovery of the control response to mescaline 28 minutes after the application of DMI. The graph at the bottom shows the time-course of the entire study (as in Fig. 13).


A
B
C
50
0
SPIKES/SEC
GLU 50
MESC 50
MIN


RESPONSE TO AGONIST
TOTAL SPIKE NUMBER (PERCENT)
200
150
100
50
0
A
B
C
MESC
GLU
0
10
20
30 MIN
DMI
(100nA,30sec)

antiserotonin action of tricyclic antidepressants (Domenjoz & Theobald, 1969).

The potentiation of responses to mescaline by desipramine is more difficult to explain. Mescaline is a monoamine which apparently has a very low affinity for uptake mechanisms in the periphery (Iversen, 1967). It would appear, then, that the uptake blockade hypothesis of potentiation (Iversen, 1974) cannot explain the potentiation by desipramine of neuronal responses to mescaline.

3:5 BIOCHEMISTRY

3:5.1 INTRODUCTION

Excitatory responses of single cortical neurones to mescaline can be potentiated by desipramine (see 3:4). It has been reported that mescaline has a very low affinity for catecholamine uptake mechanisms in the periphery (Iversen, 1967), which may imply that mescaline is not actively accumulated into nervous tissue. On this basis it was considered unlikely that the potentiation of neuronal responses to mescaline by desipramine resulted from the inhibition of the accumulation of mescaline into nerve terminals.

There are no data, however, concerning the uptake of mescaline into brain tissue. Therefore, the uptake of ^{14}C-mescaline into synaptosomes prepared from rat brain cerebral cortex was examined. As the kinetics of ^{14}C-NA accumulation into synaptosomes are well documented (see Iversen, 1974), the kinetics of ^{14}C-NA accumulation was also examined as a control. Finally, the effect of desipramine on the accumulation of ^{14}C-mescaline was examined and again parallel experiments with ^{14}C-NA served as controls.

3:5.2 THE UPTAKE OF NORADRENALINE

Table 4 shows, for different NA concentrations in the incubation medium (the substrate concentrations), the uptake velocity of NA into synaptosomes incubated at 4°C and 37°C. It can be seen that, at each NA concentration, the uptake velocity at 4°C was less than 10% of that at 37°C. Figure 34 shows the data displayed graphically.

The difference between the uptake velocities at 37°C

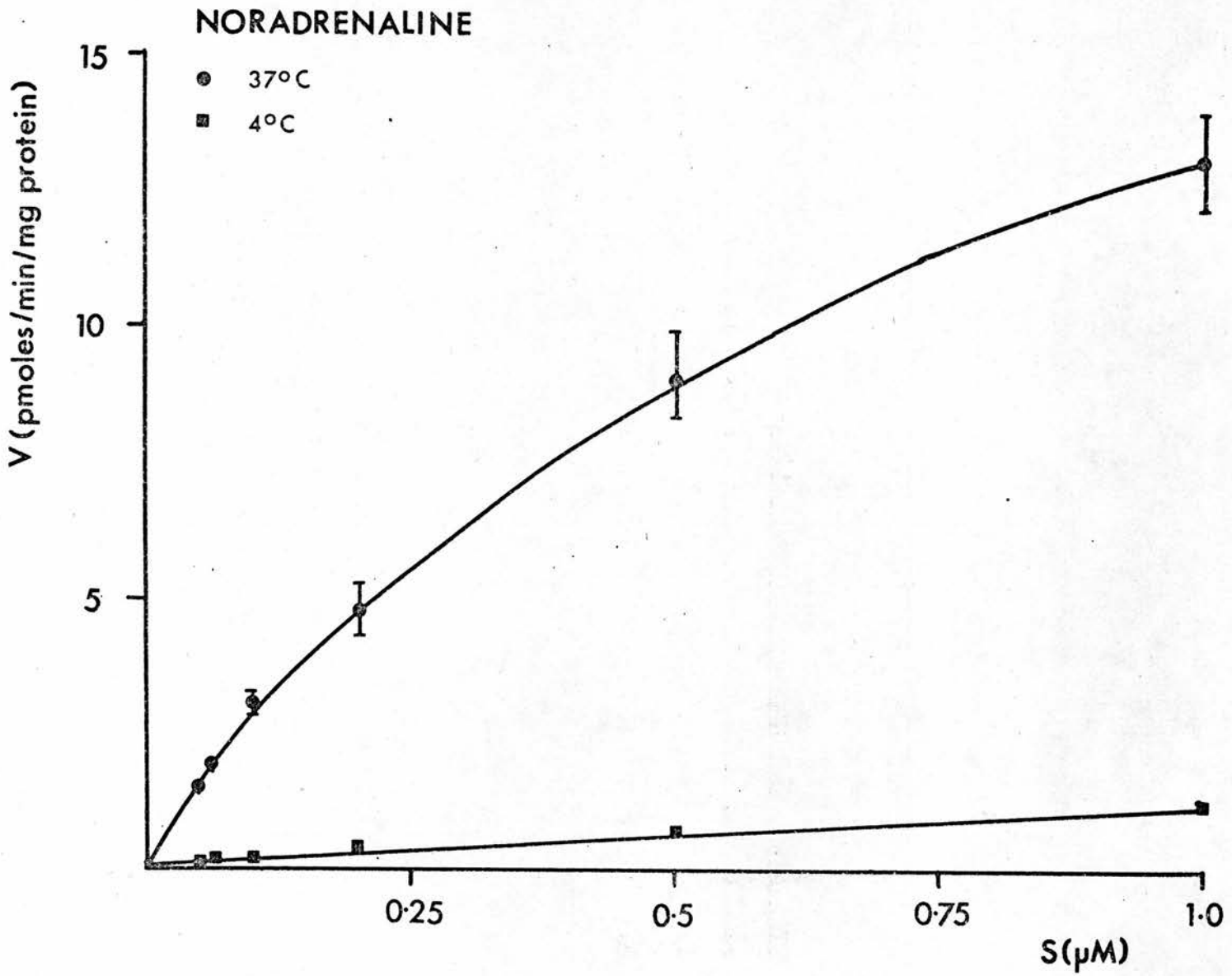
TABLE 4

The uptake of noradrenaline (NA)

NA-substrate concentration S (μM)	NA-uptake velocity V (pmoles/min/mg protein)		
	4° C	37° C	Temperature dependent
1.000	1.17 ± 0.15	13.11 ± 0.87	11.94 ± 0.87
0.500	0.71 ± 0.05	9.09 ± 0.78	8.37 ± 0.78
0.200	0.35 ± 0.02	4.81 ± 0.48	4.46 ± 0.44
0.100	0.22 ± 0.00	3.01 ± 0.09	2.79 ± 0.09
0.066	0.15 ± 0.01	1.90 ± 0.14	1.74 ± 0.14
0.050	0.11 ± 0.01	1.41 ± 0.09	1.30 ± 0.09

Each value of V is the mean (± standard error) of at least 4 observations. The temperature dependent uptake is the difference between the uptake at 37° and 4° C (Maxwell et al, 1974).

FIG. 34. The uptake of noradrenaline. Plot of the uptake velocity (V pmole/min/mg protein) against the NA-substrate concentration (S μM) for two different incubation temperatures. Each point is the mean ($\pm$ standard error) of at least 4 determinations. The lines were fitted by regression analysis.


NORADRENALINE
37°C
4°C
V (pmoles/min/mg protein)
15
10
5
0·25
0·5
0·75
1·0
S(μM)

and 4°C, for each concentration, was taken as the temperature dependent uptake velocity (Maxwell et al, 1974). Figure 36 shows for NA (closed circles) the temperature dependent uptake velocity at different NA concentrations. The graph on the left (A) is a plot of the uptake velocity (V) against the NA concentration (S). From this graph, the maximum uptake velocity (Vm) was calculated to be 19.2 ± 0.7 pmoles/min/mg protein, and the NA concentration giving use to the half-maximal uptake velocity (Km) was calculated to be 0.63 ± 0.05 µM. The graph on the right (Figure 36B, closed circles) shows the data displayed according to the method of Lineweaver and Burk (1934).

3:5.3 THE UPTAKE OF MESCALINE

As with NA, the uptake of mescaline into synaptosomes was examined at 37°C and 4°C. The uptake of mescaline into synaptosomes incubated in a sodium free medium at 37°C was also examined.

3:5.3.1. Temperature dependency of mescaline uptake

Table 5 shows, for different mescaline concentrations, (the substrate concentrations), the uptake velocity of mescaline into synaptosomes incubated at 4°C and 37°C in mammalian Ringer solution (see 2:6.1.1). It can be seen that, at each mescaline concentration, the uptake velocity at 4°C was less than 50% of that at 37°C. Figure 35 shows the data displayed graphically.

The difference between the uptake velocities at 37°C and 4°C, for each mescaline concentration, was taken as the temperature dependent uptake velocity (Maxwell et al, 1974). Figure 36 shows for mescaline (closed triangles) the temperature dependent uptake velocity at different mescaline concentrations. The graph on the left (A) is a plot of

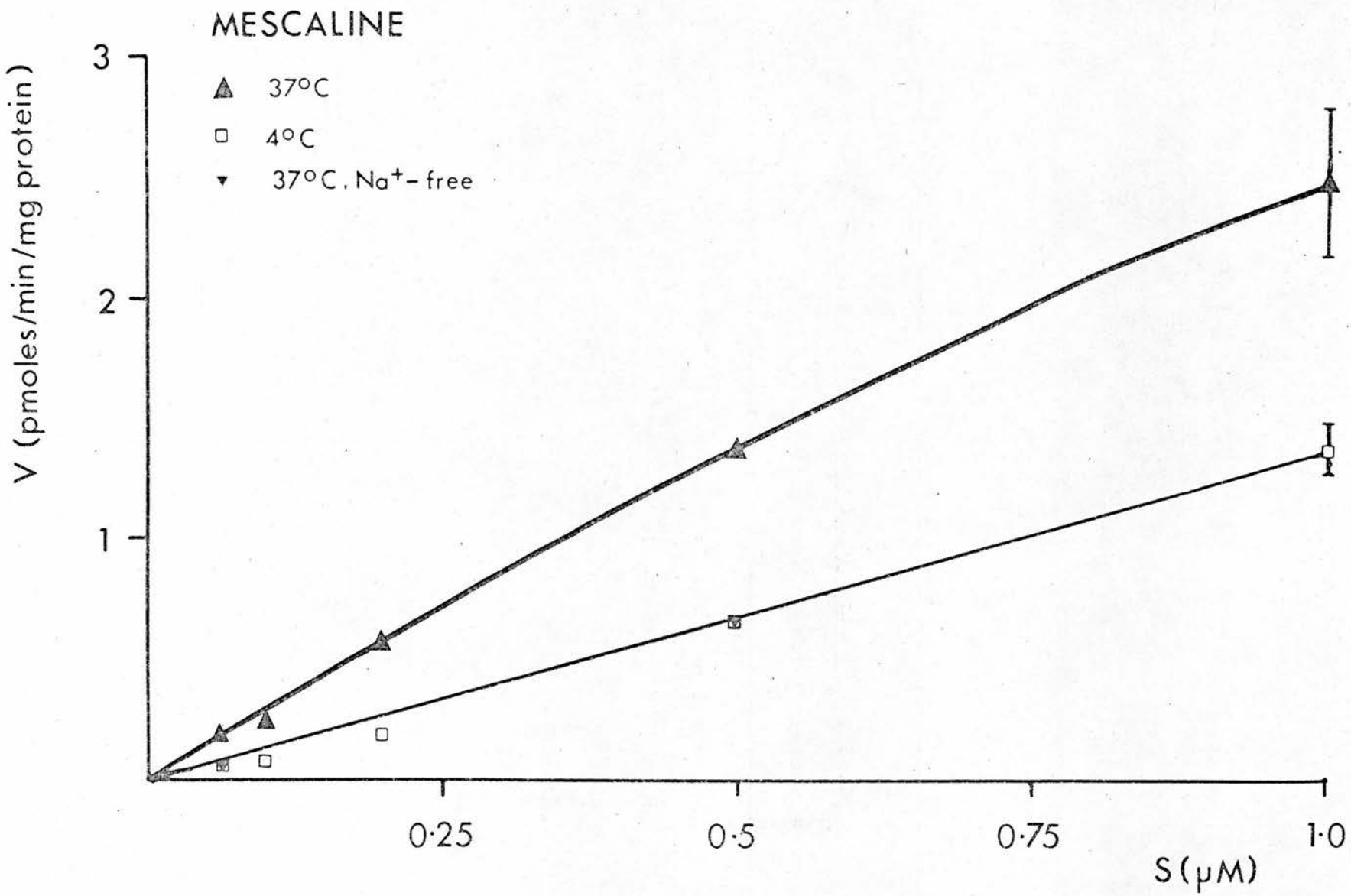
TABLE 5

The uptake of mescaline

Mescaline substrate concentration S (μM)	Mescaline uptake velocity V (pmoles/min/mg protein)		
	4° C	37° C	Temperature dependent
1.000	1.39 ± 0.13	2.53 ± 0.28	1.14 ± 0.23
0.500	0.65 ± 0.04	1.29 ± 0.07	0.73 ± 0.07
0.200	0.20 ± 0.02	0.53 ± 0.03	0.40 ± 0.03
0.100	0.09 ± 0.01	0.23 ± 0.01	0.15 ± 0.01
0.066	0.06 ± 0.01	0.21 ± 0.02	0.13 ± 0.02
0.050	0.05 ± 0.00	0.13 ± 0.01	0.08 ± 0.01

Each value of V is the mean (± standard error) of at least 4 determinations. The temperature dependent uptake is the difference between the uptake at 37 and 4° C (Maxwell et al, 1974).

FIG. 35. The uptake of mescaline. Plot of the uptake velocity (V pmoles/min/mg protein) against the mescaline substrate concentration (S/uM). Synaptosomes were incubated at 37° C in the mammalian Ringer solution, 37° C in sodium-free Ringer solution, or at 4° C in mammalian Ringer solution. Each point represents the mean (± standard error) of at least 4 determinations. The lines were fitted by regression analysis.


MESCALINE
37°C
4°C
37°C, Na+-free
V (pmoles/min/mg protein)
3
2
1
0·25
0·5
0·75
1·0
S (μM)

the uptake velocity (V) against the mescaline concentrations (S). From this graph, the maximum uptake velocity (Vm) was calculated to be 2.63 ± 0.87 pmoles/min/mg protein and the Km was calculated to be 1.24 ± 0.27 µM. The graph on the right (Figure 36B, closed triangles) shows the data displayed according to the method of Lineweaver and Burk (1923).

3:5.3.2. Sodium dependency of mescaline uptake

Table 6 compares the uptake velocity of mescaline into synaptosomes incubated in a sodium-free medium at 37°C (see 2:6.1.2) with the uptake velocity of mescaline into synaptosomes incubated in a sodium-containing medium (2:6.1.1) at both 37°C and 4°C. Removal of sodium ions from the incubation medium resulted in a 50% drop in the uptake velocity of mescaline at both mescaline concentrations tested, despite the fact that the incubation temperature was 37°C. Indeed, the uptake velocity of mescaline at 37°C in sodium-free conditions did not differ significantly from the uptake velocity of mescaline at 4°C where the sodium concentration was physiological. This was true of both mescaline concentrations tested.

3.5.4 THE EFFECT OF DESIPRAMINE ON THE UPTAKE OF NA

The effect of desipramine on the temperature dependent uptake of NA was examined. The concentration of desipramine in the incubation medium was 0.05 µM.

Table 7 shows the effect of desipramine on the uptake of NA. The data, analysed by Wilkinson's method (see 2:8.2) show that desipramine did not significantly alter the Vm for the uptake; however the apparent Km was significantly increased with respect to the control.

TABLE 6

The uptake of mescaline in sodium-free conditions

Mescaline substrate concentration S (μM)	Mescaline uptake velocity V (pmoles/min/mg protein)		
	4° C Normal Ringer	37° C Na^+-free Ringer	37° C Normal Ringer
0.500	0.65 ± 0.04	0.69 ± 0.03	1.29 ± 0.07
0.066	0.06 ± 0.01	0.08 ± 0.01	0.21 ± 0.02

Each value of V is the mean (± standard error) of at least 4 determinations. There is no statistical difference ($p > 0.02$, t test) between the uptake velocity at 4° C (normal Ringer) and the uptake velocity at 37° C (Na^+-free Ringer) for both mescaline substrate concentrations.

TABLE 7

Effect of desipramine (DMI) on the uptake of noradrenaline (NA)

NA-substrate concentration S (μM)	NA-uptake velocity V (pmoles/min/mg protein)	
	Control	DMI
1.000	11.94 ± 0.87	9.70 ± 0.87
0.500	8.37 ± 0.78	6.76 ± 0.67
0.200	4.46 ± 0.44	3.56 ± 0.37
0.100	2.79 ± 0.09	1.89 ± 0.19
0.066	1.74 ± 0.14	1.21 ± 0.12
0.050	1.30 ± 0.09	0.82 ± 0.06

Each value of V is the mean (± standard error) of at least 4 determinations.

FIG. 36. The temperature-dependent uptake of noradrenaline and mescaline.

(A) Plot of the temperature-dependent uptake velocity (V pmoles/min/mg protein) against the substrate concentration (S /uM) for noradrenaline and mescaline.

(B) Lineweaver-Burk plot of 1/V against 1/S for noradrenaline and mescaline.

Each point represents the mean (± standard error) of at least 4 determinations. All lines were fitted by regression analysis.

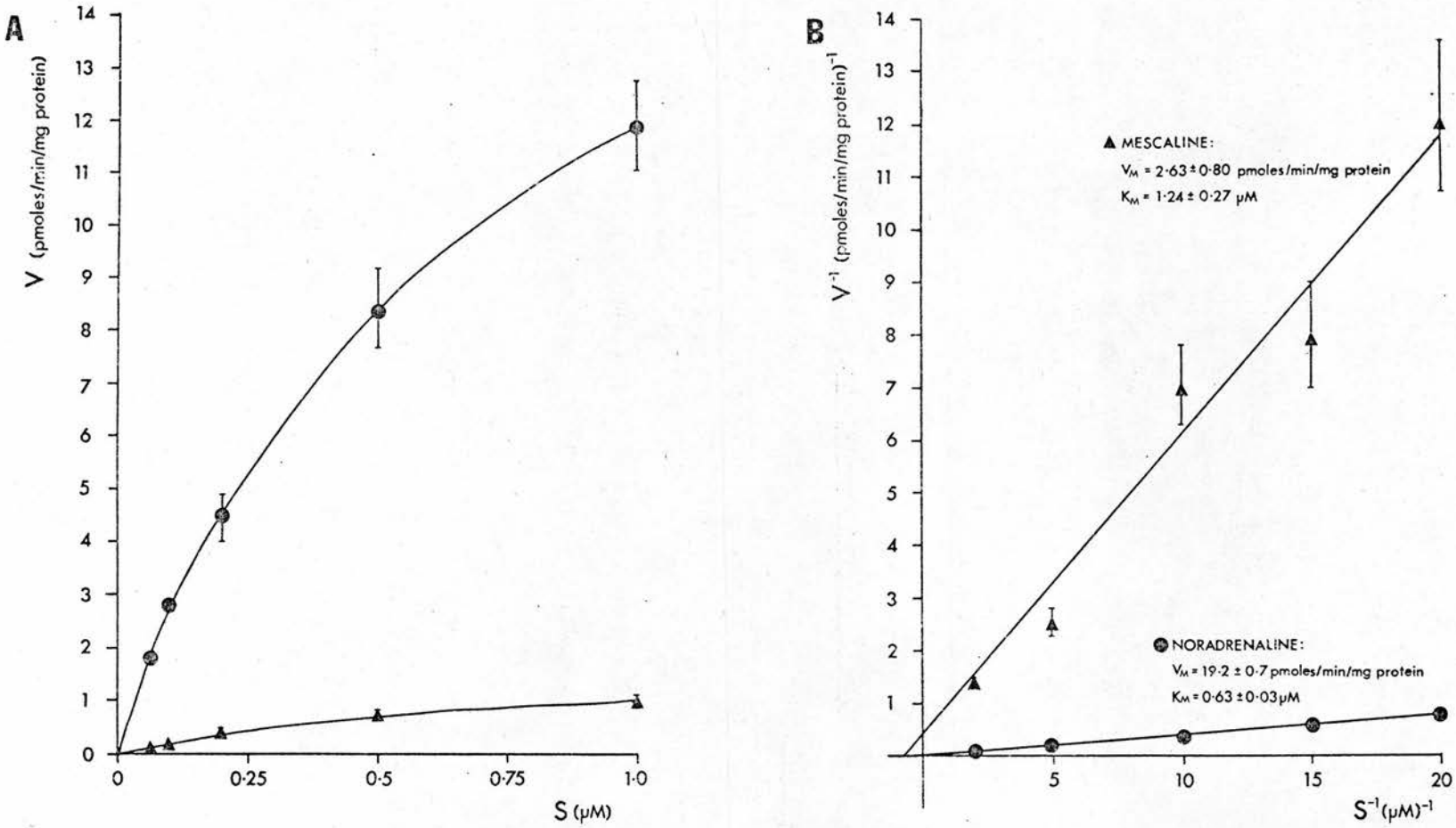

A
V (pmoles/min/mg protein)
S (μM)
0
0·25
0·5
0·75
1·0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
B
V⁻¹ (pmoles/min/mg protein)⁻¹
S⁻¹(μM)⁻¹
5
10
15
20
▲ MESCALINE:
VM = 2·63 ± 0·80 pmoles/min/mg protein
KM = 1·24 ± 0·27 μM
NORADRENALINE:
VM = 19·2 ± 0·7 pmoles/min/mg protein
KM = 0·63 ± 0·03 μM

Figure 37 shows the Lineweaver-Burk plot of the data. The open circles represent the uptake of NA in the presence of 0.05 µM desipramine and the closed circles represent the controls. Desipramine produced an increase in the slope of the line with respect to the control. However, the y-intercept (1/Vm) was not significantly altered whereas the x-intercept (1/Km) was significantly different with respect to the control. This is consistent with the criteria for competitive inhibition, and by using the analogy of enzyme kinetics (see Mahler & Cordes, 1966), the Ki for desipramine was calculated to be 0.049 $\pm$ 0.003 µM.

3:5.5 THE EFFECT OF DESIPRAMINE ON THE UPTAKE OF MESCALINE

The effect of desipramine on the temperature dependent uptake of mescaline was studied. The concentration of desipramine in the incubation medium was 0.05 µM.

Table 8 shows the effect of desipramine on the uptake of mescaline. The data, analysed by Wilkinson's method (see 2:8.2) show that desipramine did not significantly alter the Vm or Km for mescaline uptake.

Figure 38 shows the Lineweaver-Burk plot of the data. The open triangles represent the uptake of mescaline in the presence of 0.05 µM desipramine and the closed triangles represent the controls. Compared to the control, desipramine produced a small, non-significant change in the slope of the line; neither the y-intercept nor the x-intercept differed significantly from the controls.

Subsequently, the effect on mescaline uptake of increasing concentrations of desipramine was studied (see Dixon, 1953). The concentration of mescaline in the

TABLE 8

Effect of desipramine (DMI) on the uptake of mescaline

Mescaline substrate concentration S (μM)	Mescaline uptake velocity V (pmoles/min/mg protein)	
	Control	DMI
1.000	1.14 ± 0.23	1.01 ± 0.11
0.500	0.73 ± 0.07	0.67 ± 0.13
0.200	0.40 ± 0.03	0.24 ± 0.03
0.100	0.15 ± 0.01	0.14 ± 0.01
0.066	0.13 ± 0.02	0.09 ± 0.01
0.050	0.08 ± 0.01	0.08 ± 0.01

Each value of V is the mean (± standard error) of at least 4 determinations.

FIG. 37. The effect of desipramine on noradrenaline uptake. Lineweaver-Burk plots of the reciprocal of the temperature-dependent uptake velocities of NA (1/V) against the reciprocal of the NA-substrate concentration (1/S) in the presence and absence of desipramine. Each point is the mean (± standard error) of at least 4 determinations. Lines were fitted by regression analysis.

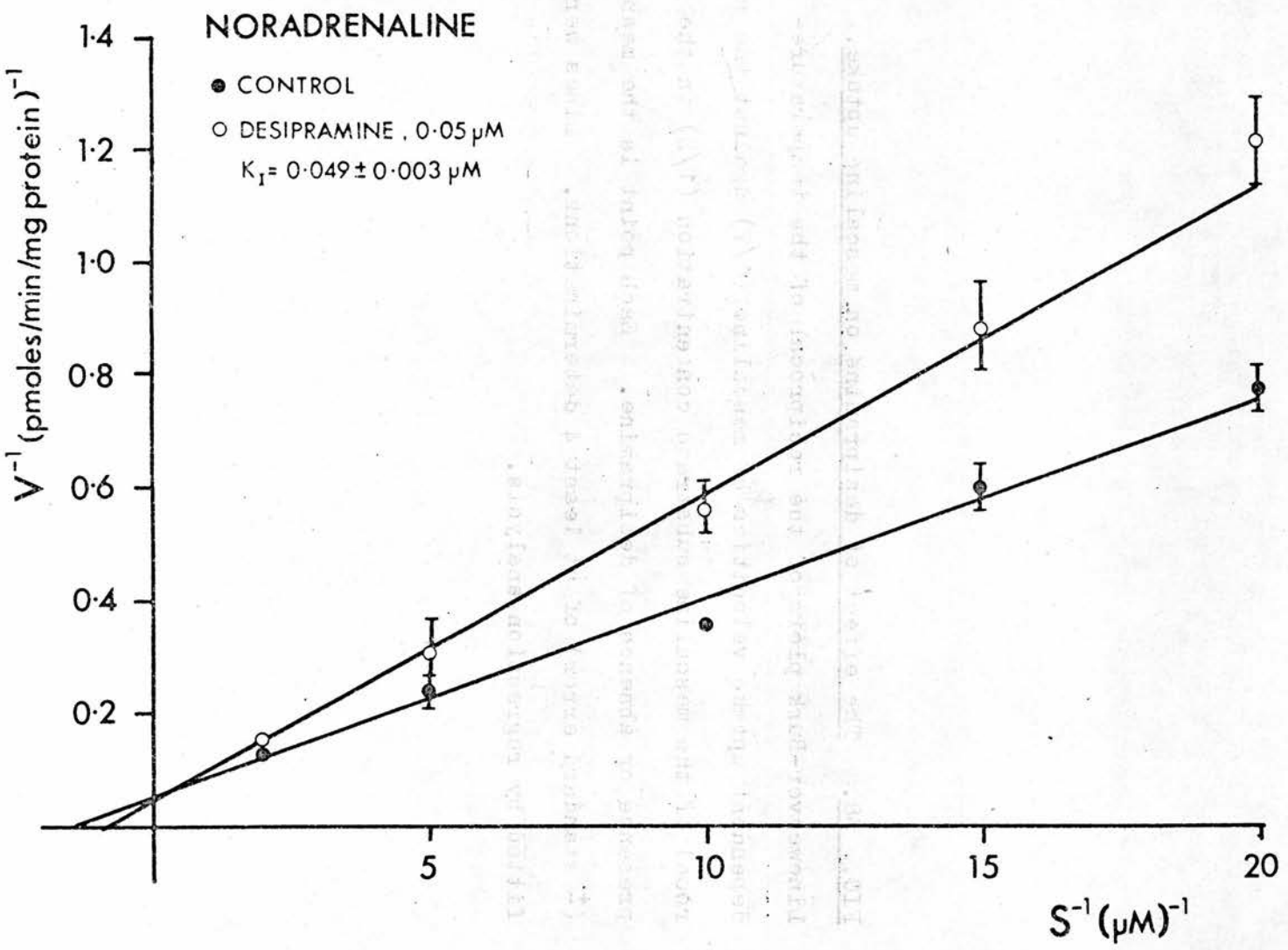
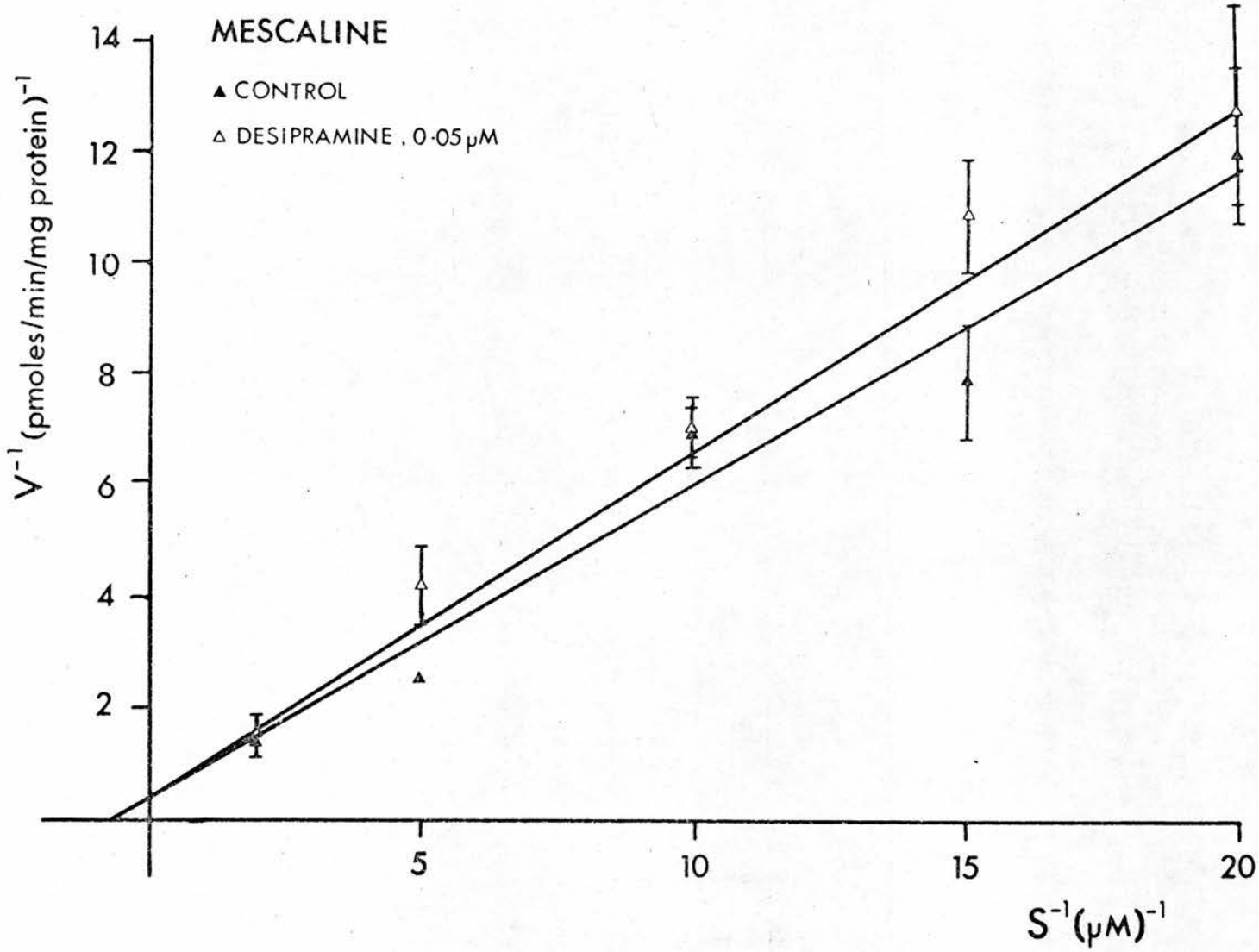

NORADRENALINE
CONTROL
DESIPRAMINE . 0·05 μM
K_I = 0·049 ± 0·003 μM
1·4
1·2
1·0
0·8
0·6
0·4
0·2
V^{-1} (pmoles/min/mg protein)$^{-1}$
5
10
15
20
S^{-1} (μM)$^{-1}$

FIG. 38. The effect of desipramine on mescaline uptake. Lineweaver-Burk plots of the reciprocal of the temperature-dependent uptake velocities of mescaline (1/V) against the reciprocal of the mescaline substrate concentration (1/S) in the presence or absence of desipramine. Each point is the mean (± standard error) of at least 4 determinations. Lines were fitted by regression analysis.


MESCALINE
▲ CONTROL
△ DESIPRAMINE . 0·05 μM
V^{-1} (pmoles/min/mg protein)$^{-1}$
14
12
10
8
6
4
2
5
10
15
20
$S^{-1}(\mu M)^{-1}$

incubation medium was either 0.1 or 1 µM. Table 9 shows, for both these concentrations of mescaline, the effect of increasing the concentration of desipramine on the uptake velocity of mescaline. There was no significant difference between the uptake velocities of mescaline at any desipramine concentration. Figure 39 is a plot of the reciprocal of the uptake velocity of mescaline against the desipramine concentration (see Dixon, 1953). The open circles represent the uptake velocity for 1 µM mescaline, whereas the closed circles represent the uptake velocity for 0.1 µM mescaline. Linear regression analysis showed the two lines to be parallel.

Thus, desipramine, within the concentration range 0.05 to 5 µM did not inhibit the temperature dependent uptake of mescaline.

3:5.6 THE EFFECT OF MESCALINE ON THE UPTAKE OF NA

The effect of mescaline on the temperature dependent uptake of ^{14}C-NA was studied. The concentration of mescaline in the incubation medium was 10 µM.

Table 10 shows the effect of mescaline on the uptake of NA. The data, analysed by Wilkinson's method (2:8.2) show that mescaline did not significantly alter the Km of NA uptake. However, the Vm of NA uptake was significantly reduced in the presence of mescaline.

Figure 40 shows the Lineweaver-Burk (1923) plot of the data. The open circles represent the uptake of NA in the presence of 10 µM mescaline and the closed circles represent the controls. The presence of mescaline increased the slope of the line with respect to the control. However, the x-intercept does not significantly differ from the

TABLE 9

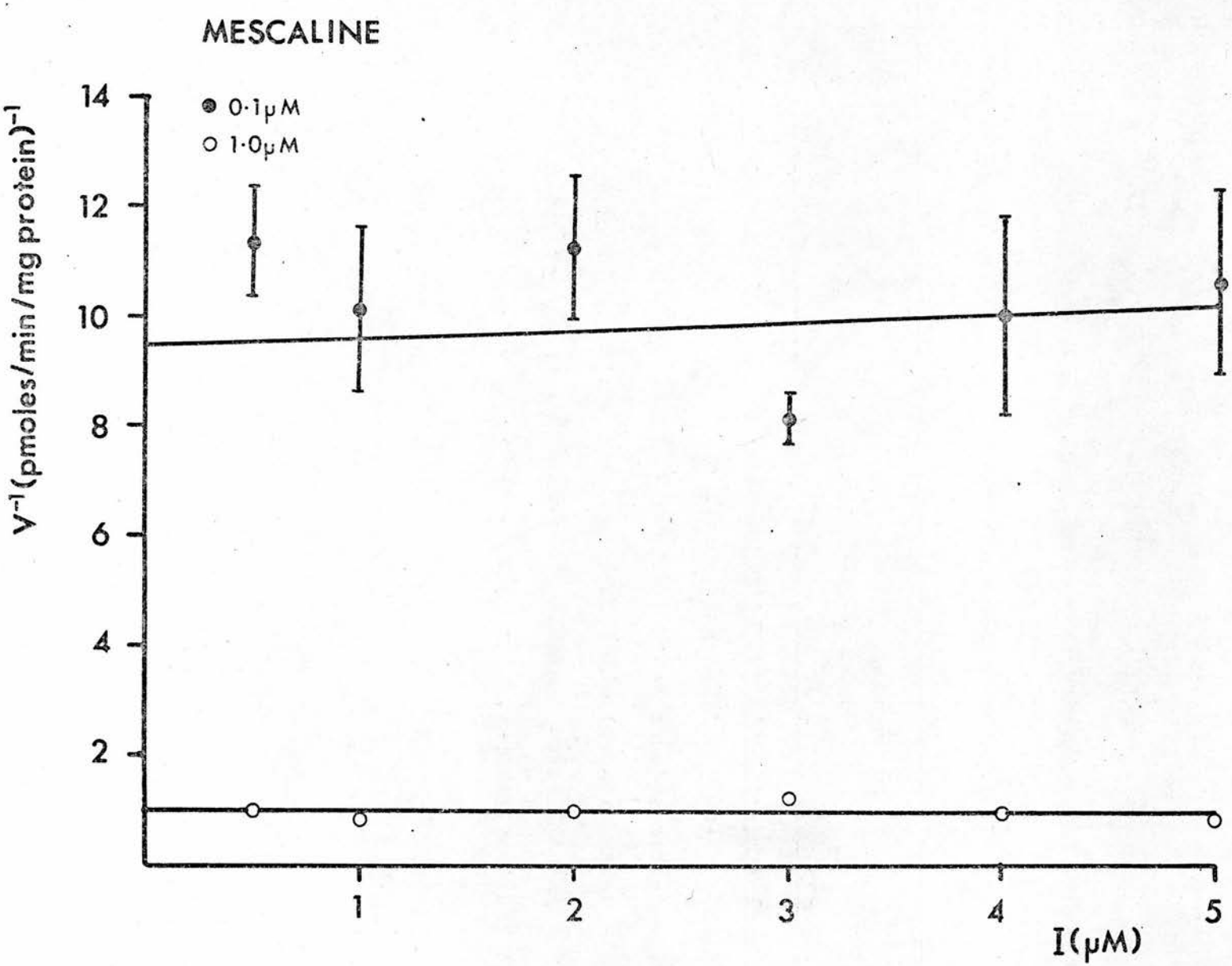
Effect of desipramine (DMI) on the uptake of mescaline

Desipramine concentration I (μM)	Mescaline uptake velocity V (pmoles/min/mg protein)	
	0.1 μM Mescaline	1 μM Mescaline
5.00	0.0937 ± 0.0126	1.261 ± 0.185
4.00	0.0993 ± 0.0152	1.139 ± 0.154
3.00	0.1230 ± 0.0067	0.883 ± 0.078
2.00	0.0885 ± 0.0091	1.000 ± 0.120
1.00	0.0988 ± 0.0127	1.175 ± 0.116
0.50	0.0878 ± 0.0073	0.963 ± 0.136
0.05	0.1381 ± 0.0119	0.994 ± 0.090

Each value of V is the mean (± standard error) of at least 4 determinations.

FIG. 39. The effect of desipramine on mescaline uptake.

Dixon plots of the reciprocal of the temperature-dependent uptake of mescaline (1/V) against the concentration of desipramine (I) in the incubation medium. The substrate concentration of mescaline in the incubation medium was either 0.1 μM or 1 μM. Each point is the mean (± standard error) of at least 4 determinations. Lines were fitted by regression analysis.


MESCALINE
0·1μM
1·0μM
14
12
10
8
6
4
2
V⁻¹(pmoles/min/mg protein)⁻¹
1
2
3
4
5
I(μM)

control whereas the y-intercept does differ significantly from the control. This is consistent with the criteria for non-competitive inhibition, and using the analogy of enzyme kinetics (see Mahler & Cordes, 1966), the Ki for mescaline was calculated to be 10.55 ± 0.81 µM.

3:5.7 DISCUSSION

The results show that NA is accumulated into synaptosomes prepared from the cerebral cortex by a temperature dependent uptake mechanism; the Km for this uptake mechanism was 0.62 µM. This is in good agreement with data published in the literature. For example, Wong, Horng and Fuller (1973) reported the Km for the active uptake of NA into synaptosomes to be 0.63 µM. Similarly, Coyle and Snyder (1969) reported that the Km for NA uptake was 0.4 µM.

Desipramine competitively inhibited the uptake of NA into synaptosomes; the apparent Km was increased whilst the Vm remained unchanged. In agreement with other authors (see Iversen, 1974), the Ki for desipramine was found to be 0.049 µM. Thus NA was accumulated into synaptosomes by an active, desipramine-sensitive process. This preparation was used to determine whether mescaline could be accumulated into synaptosomes.

There have been no studies of the accumulation of mescaline into synaptosomes in vitro. Denber and Teller (1967) found that, following the intraperitoneal injection of ^{14}C-mescaline, radioactivity was found in the brain. A small proportion of this radioactivity was associated with nerve-ending particles when the brain was subsequently homogenised (Denber & Teller, 1970). However, because the

TABLE 10

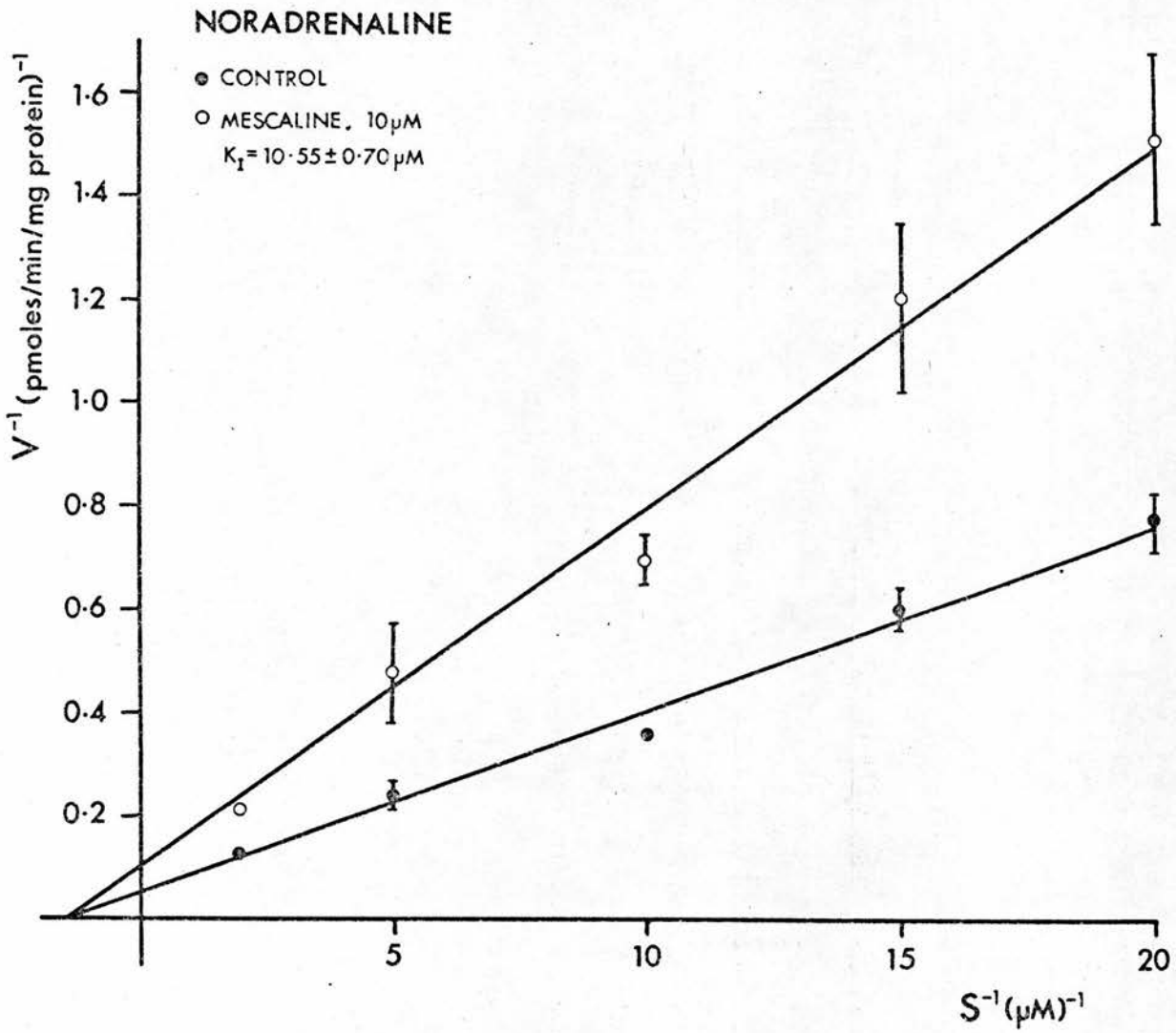
Effect of mescaline on the uptake of noradrenaline (NA)

NA-substrate concentration S (μM)	NA-uptake velocity V (pmoles/min/mg protein)	
	Control	Mescaline
1.000	11.94 ± 0.87	5.58 ± 1.07
0.500	8.37 ± 0.78	4.70 ± 0.29
0.200	4.46 ± 0.44	2.40 ± 0.45
0.100	2.79 ± 0.09	1.48 ± 0.18
0.066	1.74 ± 0.14	0.88 ± 0.09
0.050	1.30 ± 0.09	0.69 ± 0.07

Each value of V is the mean (± standard error) of at least 4 determinations.

FIG. 40. The effect of mescaline on NA uptake

Lineweaver-Burk plots of the reciprocal of the temperature-dependent uptake of NA (1/V) against the reciprocal of the NA-substrate concentration (1/S) in the presence or absence of mescaline. Each point is the mean ($\pm$ standard error) of at least 4 determinations. Lines were fitted by regression analysis.


NORADRENALINE
CONTROL
MESCALINE, 10μM
K_I = 10·55 ± 0·70 μM
V^{-1} (pmoles/min/mg protein)$^{-1}$
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
5
10
15
20
S^{-1} (μM)$^{-1}$

intraperitoneal route was used, the possibility that the radioactivity represented metabolites of mescaline could not be completely excluded (Denber & Teller, 1967).

The results presented here show that mescaline is accumulated into synaptosomes, prepared from the cerebral cortex, by a temperature and sodium dependent process. The Km for this active process was found to be 1.24 μM and the Vm was 2.63 pmoles/min/mg protein. The rate of mescaline uptake is, then, much lower than that of NA (see above; Km, 0.62 μM; Vm, 19.2 pmoles/min/mg protein).

Desipramine competitively inhibited the uptake of NA, but did not affect the uptake of mescaline into rat cerebral cortex synaptosomes. There was no change in the Km or Vm for mescaline uptake when 0.05 μM desipramine was present in the incubation medium. Indeed, no effect of desipramine on mescaline uptake could be detected even when desipramine concentrations ranging from 0.05 to 5 μM were present in the incubation medium.

It would appear, then, that the NA uptake and mescaline uptake mechanisms could be different. The NA uptake mechanism is desipramine sensitive whereas the mescaline uptake mechanism is not. More evidence for a difference between NA and mescaline uptake processes is provided by the observation that the inhibition of NA uptake by mescaline was of a non-competitive nature. If NA and mescaline were accumulated by the same uptake mechanism, one would expect mescaline to competitively inhibit the accumulation of NA (Mahler & Cordes, 1966).

The uptake of mescaline is not blocked by desipramine.

Therefore, we can conclude that the potentiation by desipramine of neuronal responses to mescaline (see 3:4) is not brought about by the blockade of mescaline uptake.

CHAPTER 4

DISCUSSION

The monoamine theory of affective disorders (see 1:2) suggests that antidepressant drugs could act by increasing the concentration of monoamine transmitters at functionally important sites in the brain. It has been suggested that tricyclic antidepressant drugs may produce such an effect by inhibiting the uptake of monoamine transmitters into presynaptic nerve terminals (Iversen, 1974). The uptake blockade hypothesis of potentiation (Iversen, 1974) thus proposes that this inhibition of monoamine uptake would be reflected in an enhanced response to monoamines of pharmacological test systems, for example, the cat nictitating membrane preparation (Sigg, 1959).

Tricyclic antidepressant drugs in fact have two effects, namely potentiation and antagonism, on monoamine responses (Callingham, 1967; Bradshaw et al, 1974). This dual effect has been attributed to two independent mechanisms. Potentiation has been explained on the basis of the blockade of monoamine uptake into nerve terminals (Herrting et al, 1961; Schildkraut, 1965; Iversen, 1974), whereas the antagonism has been related to α-receptor blockade (Turker & Khairallah, 1967). Uptake blockade, and thus potentiation, usually occurs at lower concentrations, whereas α-receptor blockade, and thus antagonism, usually occurs at higher concentration of the tricyclic antidepressant drugs (see below).

There is good evidence for this proposal derived from experiments in peripheral test systems. In the cat nictitating membrane, for example, imipramine and desipramine had a dual effect on responses to NA: small doses

potentiated whereas large doses antagonised the responses (Sturman, 1971). Moreover, in a postganglionically denervated preparation, that is, a preparation with no presynaptic terminals, no potentiation of the response could be observed; antagonism only was seen (Sturman, 1971). Similar results have been obtained using the rabbit ear artery (Callingham, 1967; McCulloch & Story, 1972) and guinea pig vas deferens (Westfall, 1973) preparations. Low doses of desipramine potentiated the contractile responses to NA whereas higher doses antagonised the responses. Once again, in the denervated preparation, only antagonism of the contractile responses could be observed (Callingham, 1967; McCulloch & Story, 1972; Westfall, 1973), indicating the importance of presynaptic terminals for potentiation.

The dual action of tricyclic antidepressant drugs on neuronal responses to monoamines has also been described in the central nervous system (Bradshaw et al, 1971a; 1974). Low doses of imipramine or desipramine potentiated the responses of single cortical neurones to NA and 5HT whereas higher doses antagonised the responses. As tricyclic antidepressant drugs block the uptake of monoamines into nerve terminals in the brain (Ross & Renyi, 1967), it is possible that uptake blockade was responsible for the observed potentiation of neuronal responses. Indeed, it has been reported that, after pretreatment with 6-hydroxydopamine, desipramine fails to potentiate responses of Purkinje cells to NA (Hoffer et al, 1971). This observation could indicate the importance of monoamine containing

terminals for the potentiation of neuronal responses.

There are, however, observations in the periphery which cannot easily be accommodated by the uptake blockade hypothesis of potentiation. Firstly, there are agonists which are not taken up into adrenergic terminals, but responses to which can be potentiated by tricyclic antidepressants. For example, it has been reported that imipramine potentiates the vasopressor response to isoprenaline (Schaeppi, 1960) when it is known that isoprenaline is not taken up into sympathetically innervated tissues (Iversen, 1967). Responses to 5HT are also potentiated by imipramine and desipramine (Gyermek & Possemato, 1960; Sigg et al, 1965) although there is no active uptake mechanism for 5HT into adrenergic terminals (Iversen, 1967). Moreover, responses of the nictitating membrane to 5HT, in contrast to NA, can be potentiated even in the sympathetically denervated preparation (Gyermek & Possemato, 1960). Secondly, there are tissues where the tricyclic antidepressants block the uptake of NA into adrenergic terminals, but fail to potentiate responses to NA. This has been reported to occur in the Auerbach's plexus longitudinal smooth muscle preparation of the guinea pig (Govier, Sugrue & Shore, 1969). Finally, there are tricyclic antidepressants which potentiate responses to NA although they are ineffective as uptake blockers. Thus the pressor response to NA is potentiated by iprindole (Gluckman & Baum, 1969) although it is known that iprindole has no effect on catecholamine uptake into tissues (Gluckman & Baum, 1969; Lahti & Maickel, 1971).

Furthermore, it has been reported by Mundo, Bonaccorsci, Bareggi, Franco, Morselli, Riva and Garattini (1974) that the potency of tricyclic antidepressant drugs in blocking catecholamine uptake and potentiating catecholamine effects do not correlate. These authors could find no correlation between the order of potencies of a range of tricyclic antidepressants in blocking NA uptake and potentiating NA responses. They concluded that inhibition of uptake was not a valid parameter for predicting a potentiating effect.

There are also observations on single neurones which cannot be explained by the uptake blockade hypothesis. It has been shown that sotalol and methysergide have a dual effect on responses to NA, 5HT and mescaline: small doses potentiate and higher doses antagonise the responses (Bevan et al, 1974a; 1974b) and it is known that methysergide is only a weak inhibitor of 5HT uptake (Born et al, 1972). It has also been shown that not only responses to monoamines, but also responses to ACh, can be modified by tricyclic antidepressant drugs; smaller doses potentiate and larger doses antagonise the responses (Bevan et al, 1973c; 1975a). As there is no evidence that ACh uptake occurs (Katz & Chase, 1971) the potentiation of responses to ACh cannot be explained on the basis of uptake blockade. Furthermore, cholinesterase inhibition cannot explain potentiation of neuronal responses to ACh (Bevan et al, 1975a).

The experiments described in this thesis have attempted to examine the validity of the uptake blockade

hypothesis of potentiation. The interaction between tricyclic antidepressant drugs and monoamine effects was examined, using the technique of microelectrophoresis, in three situations where the blockade of monoamine uptake into nerve terminals was unlikely to contribute to the effects observed. Thus, it has been shown that desipramine potentiates the responses of single caudate neurones to NA, 5HT and DA, although desipramine has been shown not to block the uptake of these amines in the caudate nucleus (Ross & Renyi, 1967; 1969). Furthermore, it has been demonstrated that iprindole can potentiate neuronal responses to NA, DA and 5HT although it is known that iprindole does not block the uptake of monoamines into brain tissue (Ross et al, 1971). Finally, the effect of desipramine on responses of single cortical neurones to mescaline was examined. An active uptake mechanism for mescaline has been demonstrated in the cerebral cortex. It has been shown that desipramine does not block the uptake of mescaline into synaptosomes. Nevertheless, responses of single cortical neurones to mescaline are potentiated by desipramine.

There are ample data, then, from both the central and peripheral nervous systems, which do not confirm the causal relationship assumed between uptake blockade and potentiation: tricyclic antidepressant drugs have been shown to potentiate monoamine effects independent of an uptake blocking action. Such results, however, cannot deny that uptake blockade may result in potentiation, but argue that uptake blockade is not a prerequisite for potentiation.

The results presented above (Chapter 3) show that the tricyclic antidepressant drugs desipramine and iprindole have a dual effect on neuronal responses to monoamines and ACh. Both antagonism and potentiation of the neuronal responses to the agonist (NA, 5HT, DA or ACh) could be observed after a brief application of the antidepressant. When both antagonism and potentiation occurred after a single application of the antidepressant, antagonism invariably preceded the development of potentiation. After a brief ejecting pulse, the concentration of the antidepressant rises quickly to a peak and then gradually declines (Castillo & Katz, 1955). Antagonism of the response is therefore likely to reflect a higher, and potentiation a lower concentration of the antidepressant. Therefore, the dual effects of antidepressant drugs on neuronal responses to monoamines and ACh appears to be dose-dependent: smaller doses potentiate and bigger doses antagonise the responses (see also Bradshaw et al, 1974; Bevan et al, 1975a).

The most plausible interpretation for the antagonism of neuronal responses by tricyclic antidepressant drugs is the blockade of postsynaptic receptors. The anti-noradrenergic, anti-serotoninergic and anticholinergic effects of tricyclic antidepressant drugs are well documented in the periphery (Domenjoz & Theobald, 1959). However, as uptake blockade is not a complete explanation for potentiation, it is possible that not only antagonism but potentiation of the responses is due to postsynaptic effects (Bevan et al, 1974a; Bradshaw et al, 1974;

Szabadi & Bradshaw, 1974; Bevan et al, 1975a). There is evidence suggesting that both excitatory and inhibitory receptors to the monoamines (Szabadi & Bradshaw, 1974) and ACh (see Bevan et al, 1975a) can coexist on the same neurone. The presence of the two kinds of receptors is often apparent in the case of biphasic responses. In the case of monophasic responses, however, the effect of one kind of receptor may be completely masked by the effect of the other (dominant) receptor which determines the direction (excitation or depression) of the observed response. Thus potentiation may be due to the selective blockade of the masked receptors by a small concentration of the antidepressant whereas antagonism may result from the blockade of both masked and dominant receptors by a higher concentration of the drug.

Consider, for example, the case of an excitatory response. It is possible that this monophasic response reflects the activation of functionally opposite masked inhibitory and dominant excitatory receptors. The activation of the inhibitory receptors would attenuate the effects of the dominant excitatory receptors. By blocking the inhibitory receptors selectively, the attenuation is removed and the response therefore potentiated. However, blocking both receptor populations results in the antagonism of the response. In this example, the antidepressant would have a higher affinity for the inhibitory receptors than for the excitatory receptors so that a low dose results in the potentiation and a high dose in the antagonism of the response. Of course, if the antidepressant has a higher

ADDITION TO 1ST PARA. P.112

When an excitatory response is potentiated or an inhibitory response is reversed, the inhibitory receptors would be selectively blocked and, conversely, when an inhibitory response is potentiated or an excitatory response is reversed, the excitatory receptors would be selectively blocked. Thus, if this hypothesis is correct, desipramine and iprindole would be more effective in blocking excitatory receptors on some neurones while on others they would be more effective against inhibitory receptors. It is difficult to explain why a drug should have, among the same group of neurones, such a dual effect on excitatory and inhibitory receptors and the hypothesis must be regarded as tentative until further experiments have been carried out.

affinity for the dominant excitatory receptors, then a low dose of the antidepressant would block the excitatory receptors selectively, unmasking the effect of the inhibitory receptors. Thus the direction of the observed response would be reversed (see for example Figure 17).

Iprindole differs from imipramine and desipramine in that it does not block the uptake of monoamines into brain tissue (Ross et al, 1971). It would appear, therefore, that the common action of antidepressant drugs is not their ability to block monoamine uptake into nervous tissue. However, all tricyclic antidepressant drugs seem to share the ability to potentiate and antagonise neuronal responses to monoamines and ACh (Table 11). In contrast to the tricyclic antidepressant drugs, the monoamine antagonists methysergide and sotalol potentiate and antagonise responses to monoamines without affecting responses to ACh (Bevan et al, 1974a; 1974b). Similarly, the cholinergic antagonist atropine potentiates and antagonises responses to ACh without affecting responses to monoamines (Johnson et al, 1969a). A common feature of the tricyclic antidepressant drugs may thus indeed be their ability to block both monoamine and ACh receptors in the brain.

As tricyclic antidepressant drugs may interact with several neurotransmitter substances, it is possible that a given concentration of an antidepressant could interact with neurotransmitters differentially. For example, the therapeutic concentration of desipramine could antagonise cholinergic transmission whilst potentiating monoamine transmission. The balance between neuronal systems could therefore be modified in the presence of the antidepressant.

TABLE 11

Effects of tricyclic antidepressants and antagonists on responses of single cortical neurones to potential neurotransmitters

	Noradrenaline	5-hydroxytryptamine	Dopamine	Acetylcholine	Glutamate
Imipramine	P, A (1)	P, A (1)	-	P, A (2)	O (1)
Desipramine	P, A (1)	P, A (1)	P, A	P, A (2)	O (1)
Iprindole	P, A	P, A	P, A	P, A	O
Sotalol	P, A (3)	P, A (3)	-	O (3)	O (4)
Methysergide	P, A (3)	P, A (3)	-	O (3)	O (5)
Atropine	O (4)	-	-	P, A (2)	O (6)

P = Potentiation; A = Antagonism; O = not affected; - = not studied.

(1) Bradshaw, Roberts & Szabadi (1974)

(2) Bevan, Bradshaw & Szabadi (1975a)

(3) Bevan, Bradshaw & Szabadi (1974b)

(4) Johnson, Roberts, Sobieszek & Straughan (1969a)

(5) Roberts & Straughan (1967)

(6) Stone (1972)

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